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Highly Oxidizing Excited States of One-Electron-Oxidized Guanine in DNA: Wavelength and pH Dependence

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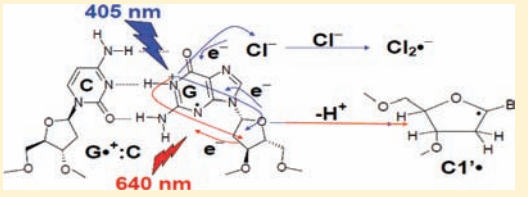
Supporting Information

ABSTRACT: Excited states of one-electron-oxidized guanine in DNA are known to induce hole transfer to the sugar moiety and on deprotonation result in neutral sugar radicals that are precursors of DNA strand breaks. This work carried out in a homogeneous aqueous glass (7.5 M LiCl) at low temperatures (77−175 K) shows the extent of photoconversion of one-electron-oxidized guanine and the associated yields of individual sugar radicals are crucially controlled by the photon energy, protonation state, and strandedness of the oligomer. In addition to sugar radical formation, highly oxidizing excited states of one-electron-oxidized guanine are produced with 405 nm light at pH 5 and below that are able to oxidize chloride ion in the surrounding solution to form $Cl_2^{\bullet-}$ via an excited-state hole transfer process. Among the various DNA model systems studied in this work, the maximum amount of $Cl_2^{\bullet-}$ is produced with ds (double-stranded) DNA, where the one-electron-oxidized guanine exists in its cation radical form ($G^{\bullet+}$:C). Thus, via excited-state hole transfer, the dsDNA is apparently able to protect itself from cation radical excited states by transfer of damage to the surrounding environment.



1. INTRODUCTION

Electronic excitation of a species often substantially increases its oxidation potential,[1,2] thereby creating an excellent one-electron-oxidizing agent. From the known values of redox potentials,[3,4] it is evident that neither the guanine cation radical ($G^{\bullet+}$), also known as the "hole", nor its deprotonated neutral form [G(−H)$^{\bullet}$] is able to oxidize a nucleoside sugar moiety via one-electron oxidation. However, our experimental and theoretical efforts have established that the photoexcitation of DNA base cation radicals forms neutral sugar radicals in DNA systems by a proton-coupled hole transfer process.[5−12,13a] The formation of sugar radicals via photoexcited base cation radicals is found to be influenced by various factors, e.g., the wavelength of the incident light, pH of the solution, length and sequence of the oligomer, and site of phosphate substitution (3′ or 5′).[5−8]

In double-stranded (ds) highly polymerized DNA, photoexcitation of one-electron-oxidized guanine produces C1′$^{\bullet}$ in the wavelength range of 310−480 nm, and no significant sugar radical formation was observed at wavelengths above 520 nm.[9] However, photoexcitation of $G^{\bullet+}$ in 2′-deoxyguanosine (dGuo) showed no wavelength dependence toward the formation of sugar radicals (C1′$^{\bullet}$, C3′$^{\bullet}$, C5′$^{\bullet}$) over the entire UVA−vis range from 330 to 800 nm.[9] Theoretical calculations employing time-dependent density functional theory (TD-DFT; B3LYP/6-31G-(d)) were carried out for $G^{\bullet+}$ in dGuo and in the dinucleoside phosphate TpdG. These calculations predicted that the light-induced excitations occurring from inner MOs to the singly occupied molecular orbital (SOMO) take place throughout the 330−880 nm range for $G^{\bullet+}$ in dGuo.[9] However, TD-DFT calculations for $G^{\bullet+}$ in TpdG[10a] predict that at longer wavelengths base-to-base hole transfer occurs whereas base-to-sugar hole transfer is predicted to occur at shorter UVA−vis wavelengths, leading to sugar radical formation after deprotonation.

To find the experimental evidence of base-to-base hole transfer via photoexcitation of $G^{\bullet+}$, these studies were extended to single-stranded (ss) DNA and dsDNA oligomers. Increasing the length of the oligomer from a 6-mer TTGGTT to a 10-mer, i.e., TTGGTTGGTT, resulted in a 50% decrease in the initial yield of the sugar radical observed after the first 20 min of the visible light exposure and a relative increase in the yield of C1′$^{\bullet}$ vs C3′$^{\bullet}$ and C5′$^{\bullet}$ found after prolonged exposure.[11]

The formation of sugar radicals via photoexcitation of one-electron-oxidized guanine in DNA and RNA monomers is found to depend on the protonation state of guanine.[8,9,13a] At pH ≥ 9, where the guanine in dGuo or in guanosine (Guo) exists in its deprotonated form [G(N1−H)$^{\bullet}$] in our system (i.e., a glassy solution at low temperature),[14a,15] only small or negligible yields of sugar radicals are found via photoexcitation.[8,9] On the other hand, in dGuo[9] or in Guo,[8] complete photoconversion of $G^{\bullet+}$ to sugar radicals occurs in the pH range (2−6) where protonated $G^{\bullet+}$ is found ($pK_a(G^{\bullet+})$ = ca. 5) in the homogeneous aqueous glass.[15]

In dsDNA oligomers containing G, facile intra-base-pair proton transfer occurs to form only G(N1−H)$^{\bullet}$:C(+H$^+$) in low-temperature (77−155 K) aqueous glasses at pH 4−6 (Scheme 1).[14a,15] Thus, from photoexcitation results obtained using monomers, no sugar radical formation is expected in dsDNA via photoexcitation of one-electron-oxidized guanine even at pH 5 and above. As expected for d[TGCGCGCA]$_2$ at pH

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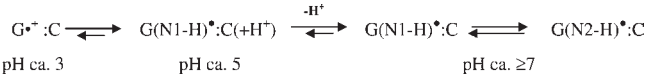
Scheme 1. Schematic Representation of the States of One-Electron-Oxidized Guanine Involved in the Protropic Equilibria in dsDNA Oligomers Observed in Our Work at Low Temperatures (77−175 K)[13a,14a a]



[a] The pH values at which the various prototropic forms of one-electron-oxidized guanine in DNA are stable in our homogeneous glassy system at low temperature (77−175 K) have been indicated.[13a] $G^{\bullet+}$:C denotes the cation radical state, whereas $G(N1-H)^{\bullet}$:C(+H^+) corresponds to the intra-base-pair proton-transferred form.[13a,14a] $G(N1-H)^{\bullet}$:C and $G(N2-H)^{\bullet}$:C denote the deprotonated states where duplex-to-solvent deprotonation occurs from the N1 atom in the guanine ring or from the exocyclic amine N atom (N2). The structures of these radicals are shown in Scheme 3.

ca. 3, the initial rate of sugar radical formation was considerable, and at pH ca. 5, the initial rate of sugar radical formation was still substantial although less than that found at pH ca. 3. Only at pH ≥ 7, at which the one-electron-oxidized GC base pair exists in the deprotonated state [as $G(N1-H)^{\bullet}$:C and $G(N2-H)^{\bullet}$:C], was the initial rate of formation of sugar radicals found to be very low.[13a] These experimental observations have led to the hypothesis that formation of a sugar radical requires the one-electron-oxidized GC base pair to be in its cation radical state ($G^{\bullet+}$:C),[13a] or for $G(N1-H)^{\bullet}$:C(+H^+), the reprotonation of the guanine base in the excited state is proposed to precede the deprotonation from the sugar moiety.[13a] In homogeneous aqueous glasses at 175 K, at pH 7−9, one-electron-oxidized G in d[TGCGCGCA]$_2$ is reported to exist in an equilibrium mixture of the $G(N2-H)^{\bullet}$:C and $G(N1-H)^{\bullet}$:C forms.[13a] Thus, for the formation of a sugar radical via photoexcitation of either $G(N1-H)^{\bullet}$:C or $G(N2-H)^{\bullet}$:C, the proton must return from the surrounding solvent to reprotonate the guanine, which explains the lack of significant sugar radical formation on photoexcitation.

Our previous experimental work has shown that a wavelength dependence exists for the formation of sugar radicals in ssDNA oligomers,[11] d[TGCGCGCA]$_2$,[13a] and highly polymerized DNA.[9] In this work, we present evidence that photoexcitation of one-electron-oxidized guanine in the cation radical state ($G^{\bullet+}$) in monomers (dGuo) or in ssDNA or dsDNA oligomers oxidizes the surrounding matrix (a homogeneous 7.5 M LiCl solution), thereby resulting in the formation of $Cl_2^{\bullet-}$ in a wavelength- and pH-dependent manner. Our results suggest that the reduction potential of excited $G^{\bullet+}$ is higher than that of excited $G(N1-H)^{\bullet}$ as well as that of the ground-state reduction potential of $Cl_2^{\bullet-}$. Furthermore, for dsDNA at pH 5 and below, employing dsDNA oligomers (8-mer d[TGCGCGCA]$_2$ and 16-mer d[TATAGCGGCCGCTATA]$_2$) and lasers at wavelengths of 405 and 640 nm in this work, we show that the formation of specific sugar radicals formed via photoexcitation is dependent on the wavelength of the incident light; specially, in dGuo, we find increased formation of C5′• at 405 nm with a decrease in the yield of C1′• owing to the photoconversion of C1′• to C5′• at 405 nm.

2. MATERIALS AND METHODS

2.1. Compounds. dGuo was obtained from Sigma Chemical Co. (St. Louis, MO). The ssDNA oligomer TGT and the dsDNA oligomers d[TGCGCGCA]$_2$ and d[TATAGCGGCCGCTATA]$_2$ were procured from SynGen, Inc. (Hayward, CA). These DNA oligomers were lyophilized, desalted, and column purified.

Potassium persulfate (crystal) was obtained from Mallinckrodt, Inc. (Paris, KY), and lithium chloride (99% anhydrous, Sigma Ultra) was purchased from Sigma. Deuterium oxide (99.9 atom % D) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). All compounds were used without any further purification.

2.2. Preparation of Solutions. As per our earlier work with the monomers,[8,9,12−16] ∼3 mg of dGuo was dissolved in 1 mL of 7.5 M LiCl in D_2O in the presence of 5−6 mg of $K_2S_2O_8$ as the electron scavenger. Following our ongoing work regarding DNA oligomers,[10,11,13a,14,16] the homogeneous solutions of TGT and of the dsDNA oligomers were prepared by dissolving ca. 1.5 mg of compound in 0.50 mL of 7.5 M LiCl/D_2O in the presence of 4−5 mg of $K_2S_2O_8$ as the electron scavenger.

The use of $K_2S_2O_8$ as the electron scavenger enabled us to study only the one-electron-oxidized radicals.

Our previous work showed that the dsDNA 8-mer d[TGCGCGCA]$_2$ is double-stranded in 7.5 M LiCl in D_2O up to 48 °C.[13a] We note that, in homogeneous aqueous (H_2O) glasses (10 M LiCl), the dsDNA oligomers have been reported to be in the B-conformation.[13b]

2.3. Adjustment of the pH and pD. Following our work,[8,9,12−16] the pH/pD values of the solutions of dGuo, TGT, and the dsDNA oligomers mentioned above were adjusted by quickly adding the adequate micromole amounts of 0.1−1 M NaOH in D_2O and 0.1−1 M HCl in D_2O under ice-cooled conditions. Owing to the high ionic strength (7.5 M LiCl) of these solutions and also due to the use of pH papers, all the pH/pD values reported in this work are approximate.

Subsequently, these homogeneous solutions were degassed by bubbling thoroughly with nitrogen gas at room temperature.

2.4. Preparation of Glassy Samples and Their Storage. Following our work,[8−16] the homogeneous solutions of dGuo, TGT, and the dsDNA oligomers were drawn into 4 mm Suprasil quartz tubes (catalog no. 734-PQ-8, Wilmad Glass Co., Inc., Buena, NJ) and these tubes containing these solutions were rapidly cooled to 77 K. This rapid cooling of these liquid solutions resulted in transparent glassy solutions which were later used for the irradiation, annealing, and subsequent photoexcitation experiments.

All samples were stored at 77 K in Teflon containers in the dark.

2.5. γ-Irradiation of Glassy Samples and Their Storage. As per our work,[13a] the Teflon containers with these transparent glassy samples were placed in a 400 mL Styrofoam Dewar under liquid nitrogen (77 K). The γ-irradiation of the glassy samples was performed at 77 K with the help of the 109-GR 9 irradiator, which contains a properly shielded ^{60}Co γ source.

The glassy samples of these DNA oligomers were γ-irradiated with an absorbed dose of 2.5 kGy.

2.6. Annealing of the Samples. Following our previous work with dGuo[9,14,15] and TGT,[11] the γ-irradiated glassy samples of dGuo and TGT used in this study were annealed at 155 K for 15−20 min. Following our work regarding DNA oligomers,[13a,14a] the annealing studies of the γ-irradiated glassy samples of the dsDNA oligomers were carried out in the range of 155−175 K.

The annealing studies for all these above-mentioned samples were carried out by using a variable-temperature assembly (Air Products) via cooled nitrogen gas in the dark which regulated the gas temperature within 4 °C. To monitor the sample temperature during annealing, we used a copper−constantan thermocouple in direct contact with the sample.

The annealing of the sample softens the glass, which allows the radicals, e.g., $Cl_2^{\bullet-}$ to migrate and react with solute to produce one-electron-oxidized guanine.[9]

2.7. Photoexcitation. For photoexcitation experiments, a thermoelectrically cooled blue laser (TECBL-30G-405, World Star Technologies, Toronto, lot 6880, λ = 405 nm, 30 mW) and a red laser (UH5-40G-

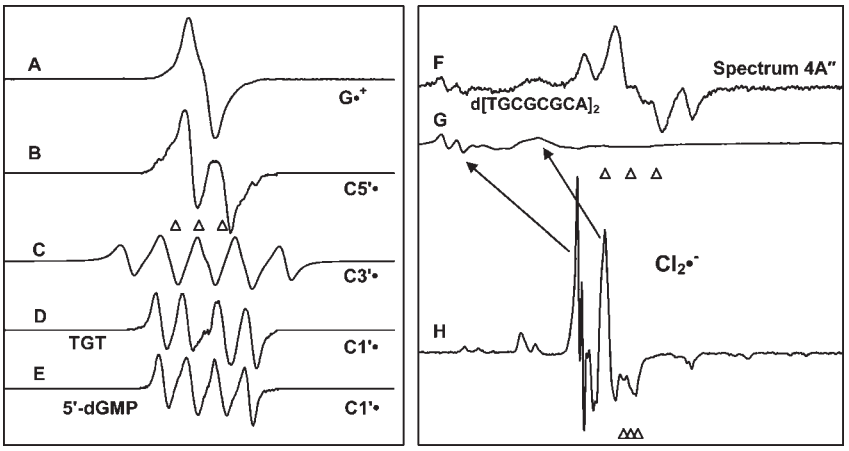


Figure 1. Benchmark spectra used for computer analysis. (A) $G^{\bullet+}$ produced from dGuo via one-electron oxidation by $Cl_2^{\bullet-}$ at pD ca. 3.[14,15] (B) C5′• formed via photoexcitation of $G^{\bullet+}$ in 8-D-3′-dGMP.[9] (C) C3′• produced via photoexcitation of $G^{\bullet+}$ in dGuo,[9] (D) C1′• produced via photoexcitation of $G^{\bullet+}$ in TGT.[11] (E) C1′• produced via photoexcitation of $G^{\bullet+}$ in 5′-dGMP.[9] (F) As an example of the formation of the matrix radical ($Cl_2^{\bullet-}$) owing to 405 nm photoexcitation of one-electron-oxidized G (hole transfer to the matrix), spectrum A″ from Figure 4 is shown here. (G) Partial spectrum (220 G scan) of the total $Cl_2^{\bullet-}$ spectrum (1000 G scan) shown in (H). The scan range of the $Cl_2^{\bullet-}$ spectrum shown in (G) matches those of (A)−(E). (H) $Cl_2^{\bullet-}$ spectrum obtained via γ-irradiation of 7.5 M LiCl/D_2O solution containing persulfate to capture electrons followed by annealing at 143 K for 20 min.[18] The three reference markers (open triangles) in this figure and in other figures show the position of Fremy's salt resonance with the central marker at g = 2.0056. Each of these markers is separated from the others by 13.09 G.

640C, World Star Technologies, lot 6880, λ = 640 nm, 40 mW) were used as light sources.

Following our work regarding photoexcitation,[8−16] the same temperature assembly that had already been used to maintain the temperatures during the annealing processes was also employed to maintain the temperature during the photoexcitation of these glassy samples. The temperature during photoexcitation was maintained at 143 ± 2 K.

2.8. Electron Spin Resonance. Following γ-irradiation, annealing, and photoexcitation using lasers of different wavelengths, the glassy samples were immersed immediately in liquid nitrogen, and an electron spin resonance (ESR) spectrum was recorded at 77 K and at 45 dB (6.3 μW). Fremy's salt (with g(center) = 2.0056 and A_N = 13.09 G) was used for field calibration.[17] We recorded these ESR spectra using a Varian Century Series ESR spectrometer operating at 9.3 GHz with an E-4531 dual-cavity, 9 in. magnet and with a 200 mW klystron.[17] All the ESR spectra were recorded at 77 K.

2.9. Analyses of Experimental ESR Spectra. The fractional compositions of radicals used as benchmarks (Figure 1) in the experimentally recorded ESR spectrum were determined with the help of least-squares fitting using programs, viz., ESRPLAY and ESRADSUB, developed in our laboratory. By employing the doubly integrated areas of the benchmark spectra, we estimated the percentage (or fractional) contribution of a particular radical in the experimentally found ESR spectrum. The number of spins of each radical is directly proportional to the doubly integrated area in its ESR spectrum.

The benchmark spectra of the radicals that were used to analyze the experimental spectra recorded after annealing and after photoexcitations using lasers are shown in Figure 1.

In Figure 1, spectrum A is the benchmark spectrum of $G^{\bullet+}$ obtained via one-electron oxidation by $Cl_2^{\bullet-}$ in dGuo at pD ca. 3 in 7.5 M LiCl/D_2O.[14,15] The ESR spectra of C5′• (Figure 1B) and of C3′• (Figure 1C) were obtained via photoexcitation of $G^{\bullet+}$ in dGuo in 7.5 M LiCl/D_2O (pD ca. 5).[9]

Two benchmark spectra of C1′• have been used to determine the fraction of C1′• present in the experimentally recorded spectra (Figures 2−5). The C1′• spectrum shown in Figure 1D was obtained from TGT,[11] whereas the C1′• spectrum presented in Figure 1E was from 5′-dGMP.[9] The β-hyperfine couplings of C2′−H atoms for C1′• varied slightly in these two spectra (16 G (1 βH) and 32 G (1 βH) for C1′• from 5′-dGMP[9] and 15 G (1 βH) and 34.5 G (1 βH) for C1′• from TGT[11]). Spectrum D was used to analyze the percentage contribution of C1′• in the experimental spectra obtained from TGT (Figure 3) and from the dsDNA oligomers (Figures 4 and 5). On the other hand, spectrum E was employed to analyze the percentage contribution of C1′• in the experimental spectrum (Figure 2) obtained from the dGuo samples. For spectra A−E, the three ESR lines (shown as open triangles) of Fremy's salt are each separated by 13.09 G with g(center) = 2.0056. All the spectra (Figures 1−5 along with Supporting Information Figures S1 and S2) are stored as 1000-point arrays, and the Fremy field markers in all these spectra are at 440, 500, and 560, except for the spectrum shown in Figure 1H, in which they are at 487, 500, and 513.

In this work, we have found that 405 nm photoexcitation of one-electron-oxidized G results in hole transfer to the matrix, resulting in the formation of $Cl_2^{\bullet-}$ (see Figures 2−5). In Figure 1F, we have taken spectrum A″ from Figure 4 (i.e., 405 nm photoexcitation of one-electron-oxidized d[TGCGCGCA]$_2$) as an example of the formation of the matrix radical ($Cl_2^{\bullet-}$). In Figure 1H, we present the full spectrum of $Cl_2^{\bullet-}$ over 1000 G as shown in previous work.[18] Hence, comparison of spectrum G (partial $Cl_2^{\bullet-}$ spectrum) with spectrum H (complete $Cl_2^{\bullet-}$ spectrum) shows that only the two large low-field resonances from $Cl_2^{\bullet-}$ are observed in spectrum G, and these are indicated by arrows. To analyze the percentage composition of each sugar radical in Figures 2−5, we have subtracted the $Cl_2^{\bullet-}$ line components by employing the $Cl_2^{\bullet-}$ spectrum presented in Figure 1G (partial $Cl_2^{\bullet-}$ spectrum over the same scan range as spectra A−F (220 G scan)). To analyze the percentage of $Cl_2^{\bullet-}$ produced via 405 nm photoexcitation of one-electron-oxidized G, for example, in spectrum F, we simulated spectra that matched the height of the large low-field resonances from $Cl_2^{\bullet-}$ (spectrum H).

The structures of the various sugar radicals and the DNA base radicals studied in this work are shown in Schemes 2 and 3, respectively.

3. RESULTS AND DISCUSSION

3.1. Protonation States of the One-Electron-Oxidized Compounds Studied at pD Values of ca. 3, 5, and 9. *3.1.1. dGuo.* In Figure 2 A−C, the ESR spectra of one-electron-oxidized guanine in dGuo formed via thermal annealing of the

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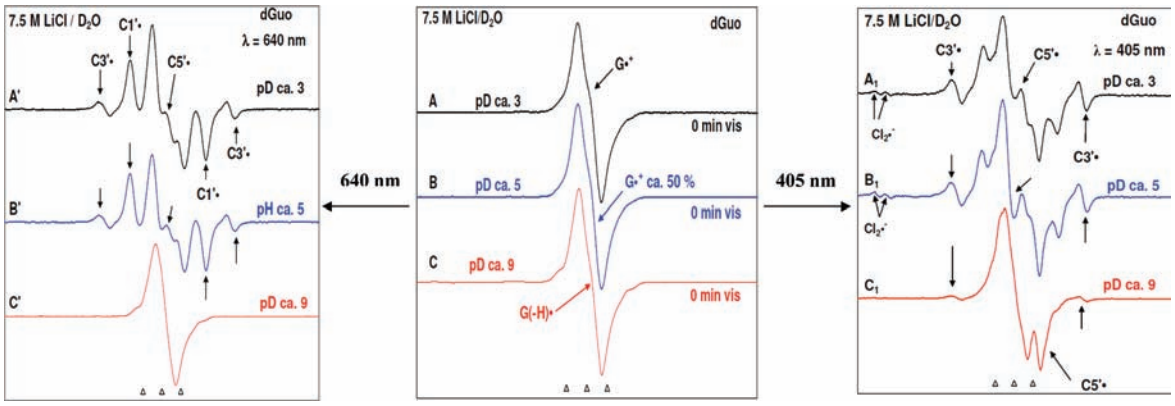

Figure 2. ESR spectra of one-electron-oxidized dGuo formed by the attack of $Cl_2^{\bullet-}$ in the presence of electron scavenger $K_2S_2O_8$ in 7.5 M LiCl/D_2O at (A) pD ca. 3, (B) pD ca. 5, and (C) pD ca. 9. After exposure of these samples to the 405 nm laser (right panel) for 1 h (spectra A_1 and B_1) and to the 640 nm laser (left panel) for 3 h 30 min (spectrum A′) and 5 h 30 min (spectrum B′), the formation of sugar radicals is observed, mainly C5′• and C3′• along with the matrix radical $Cl_2^{\bullet-}$ (with the 405 nm laser) and C5′•, C3′•, and C1′• (with the 640 nm laser). No sugar radicals are observed at pD ca. 9 with the 640 nm laser, but a small amount (ca. 20%) is observed with the 405 nm laser for 30 min.

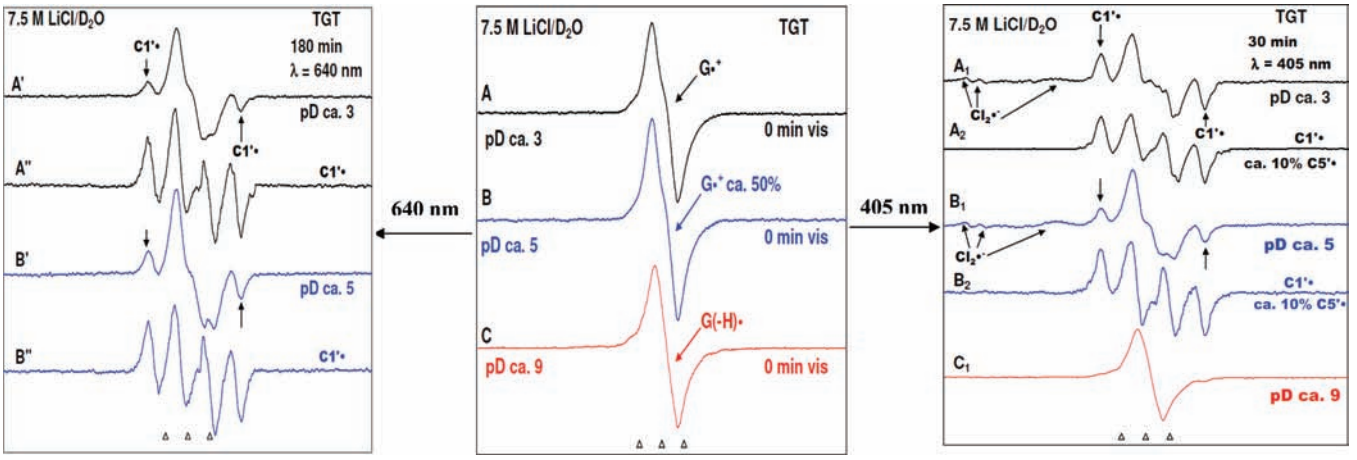

Figure 3. ESR spectra of one-electron-oxidized TGT (3 mg/mL) formed by attack of $Cl_2^{\bullet-}$ via thermal annealing of the sample at 155 K in the presence of electron scavenger $K_2S_2O_8$ in 7.5 M LiCl/D_2O (A) at pD ca. 3, (B) at pD ca. 5, and (C) at pD ca. 9. The spectra were obtained after exposure of duplicate samples at each pD to a 640 nm (40 mW) laser (left panel) for 3−5 h and to a 405 nm (30 mW) laser (right panel) for 30 min at 143 K. Photoexcitation of one-electron-oxidized guanine in TGT with both the 405 and 640 nm lasers leads to loss of one-electron-oxidized guanine with the concomitant formation of sugar radicals, and only in the case of 405 nm photoexcitation is formation of the matrix radical ($Cl_2^{\bullet-}$) observed. Subtraction of matrix radicals ($Cl_2^{\bullet-}$) and the remaining one-electron-oxidized guanine (ca. 25%) results in spectra A_2 and B_2, which show the formation of mainly C1′• and a very small amount of C5′•. No photoconversion from G(−H)• is found at pD ca. 9 as shown in spectrum C_1. Spectra A″ and B″ (left panel) are obtained after the subtraction of the remaining one-electron-oxidized guanine, $G^{\bullet+}$ (ca. 65%), which shows the predominant formation of C1′•.

sample at 155 K in the presence of electron scavenger $K_2S_2O_8$ in 7.5 M LiCl glass are shown. As reported earlier,[14,15] one-electron-oxidized guanine in dGuo at pD ca. 3 exists in its "pristine" cation radical form ($G^{\bullet+}$) (spectrum A, black), at the native pD of 7.5 M LiCl/D_2O (pD ca. 5) it exists half in its protonated form ($G^{\bullet+}$) and half in its deprotonated form (G(N1−H)• or G(−H)•) (spectrum B, blue), and at pD ca. 9 it exists in the deprotonated form (G(−H)•) (spectrum C, red).

3.1.2. TGT. In Figure 3A−C, the ESR spectra of one-electron-oxidized TGT at different pD values of ca. 3, 5, and 9 in 7.5 M LiCl/D_2O are shown. The one-electron oxidation of TGT is carried out by $Cl_2^{\bullet-}$ via thermal annealing of the sample at 155 K. Spectrum A is found to be identical to the $G^{\bullet+}$ spectrum (Figure 2A, black) in dGuo. Using the benchmark spectrum shown in Figure 1A and also from our previous study using 8-deuterioguanine-incorporated TGT,[14] it is clear that one-electron-oxidized guanine in TGT exists in the cation radical form ($G^{\bullet+}$) at pD ca. 3 (spectrum A, black), in equal amounts of its $G^{\bullet+}$ and G(−H)• forms at pD ca. 5 (for simplicity, it will be referred to as $G^{\bullet+}$ at this pD) (spectrum B, blue), and in its deprotonated form (G(−H)•) at pD ca. 9 (spectrum C, red).

3.1.3. d[TGCGCGCA]$_2$. The ESR spectra of one-electron oxidized d[TGCGCGCA]$_2$ at different pD values of ca. 3 and 9 in 7.5 M LiCl/D_2O are presented in Figure 4A−C. The one-electron oxidation of d[TGCGCGCA]$_2$ was carried out by $Cl_2^{\bullet-}$ via thermal annealing of the sample in the temperature range from 155 to 175 K. Spectrum A of one-electron-oxidized d[TGCGCGCA]$_2$ at pD ca. 3 matches that of the $G^{\bullet+}$ benchmark spectrum (spectrum A of Figure 1). Our previous work which established the nature of protonation states vs pH in dsDNA oligos[13a,14a] allows us to assign one-electron-oxidized d[TGCGCGCA]$_2$ to exist in the following forms: pD 3, cation

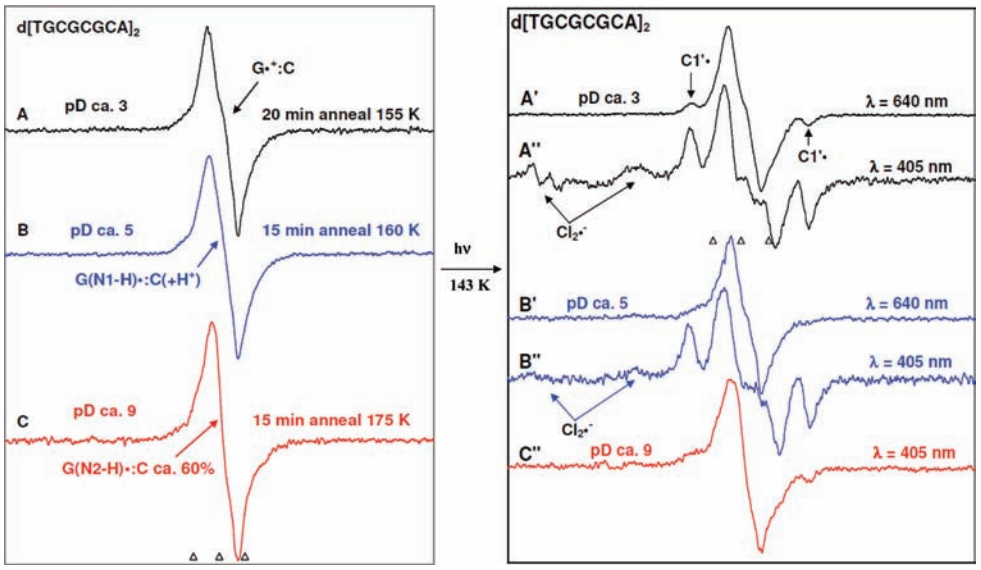

Figure 4. Left panel: ESR spectra of one-electron-oxidized d[TGCGCGCA]$_2$ (3 mg/mL) formed via one-electron oxidation by $Cl_2^{\bullet-}$ via thermal annealing of the sample in the temperature range of 155−175 K in the presence of electron scavenger $K_2S_2O_8$ in 7.5 M LiCl/D_2O. Spectrum A (black) at pD ca. 3 is assigned to the cation radical state ($G^{\bullet+}$:C), spectrum B (blue) at pD ca. 5 to the intra-base-pair proton-transferred state (G(N1−H)$^{\bullet}$:C(+H$^+$)), and spectrum C (red) at pD ca. 9 to the deprotonated states G(N2−H)$^{\bullet}$:C (ca. 60%) and G(N1−H)$^{\bullet}$:C (ca. 40%).[13a] Right panel: Spectra obtained after exposure of duplicate samples at each pD to a 640 nm (40 mW) laser for 1 h (spectrum A′, black, pD ca. 3; spectrum B′, blue, pD ca. 5) and to a 405 nm (30 mW) laser for 30 min (spectrum A″, black, pD ca. 3; spectrum B″, blue, pD ca. 5; spectrum C″, red, pD ca. 9) at 143 K. Photoexcitation of one-electron-oxidized guanine in d[TGCGCGCA]$_2$ at pD values of ca. 3 and 5 using the 405 nm laser leads to loss of one-electron-oxidized guanine (20% remaining) with the concomitant formation of sugar radicals having predominantly C1′$^{\bullet}$ and a small extent (ca. 5%) of C5′$^{\bullet}$. Only in the case of 405 nm photoexcitation is the formation of the matrix radical ($Cl_2^{\bullet-}$) observed. No photoconversion is found at pD ca. 9 as shown in spectrum C″. Spectrum A′ (pD ca. 3) shows the small amount (ca. 15%) of formation of C1′$^{\bullet}$ via 640 nm photoexcitation of the cation radical state ($G^{\bullet+}$:C), whereas spectrum B′ shows very little photoconversion at pD ca. 5.

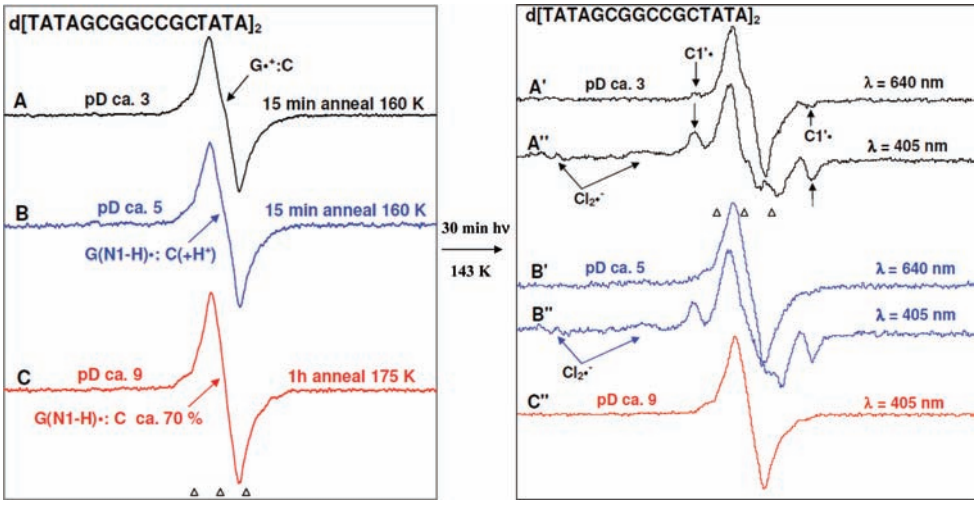

Figure 5. Left panel: ESR spectra of one-electron-oxidized d[TATAGCGGCCGCTATA]$_2$ (3 mg/mL) formed via one-electron oxidation by $Cl_2^{\bullet-}$ via thermal annealing of the sample in the temperature range of 155−175 K in the presence of electron scavenger $K_2S_2O_8$ in 7.5 M LiCl/D_2O. Spectrum A (black) at pD ca. 3 is assigned to the cation radical state ($G^{\bullet+}$:C), spectrum B (blue) at pD ca. 5 to the intra-base-pair proton-transferred state (G(N1−H)$^{\bullet}$:C(+H$^+$)), and spectrum C (red) at pD ca. 9 to the deprotonated states G(N1−H)$^{\bullet}$:C (ca. 70%) and G(N2−H)$^{\bullet}$:C (ca. 30%) (see Supporting Information Figure S1). Right panel: Spectra obtained after exposure of duplicate samples at each pD to a 640 nm (40 mW) laser for 30 min (spectrum A′, black, pD ca. 3; spectrum B′, blue, pD ca. 5) and to a 405 nm (30 mW) laser for 30 min (spectrum A″, black, pD ca. 3; spectrum B″, blue, pD ca. 5; spectrum C″, red, pD ca. 9) at 143 K. Photoexcitation of one-electron-oxidized guanine in d[TGCGCGCA]$_2$ at pD values of ca. 3 and 5 using the 405 nm laser leads to loss of one-electron-oxidized guanine (35−40% remaining) with the concomitant formation of sugar radicals having predominantly C1′$^{\bullet}$ and a small extent (ca. 5%) of C5′$^{\bullet}$. Only in the case of 405 nm photoexcitation is the formation of the matrix radical ($Cl_2^{\bullet-}$) observed. No photoconversion is found at pD ca. 9 as shown in spectrum C″. Spectrum A′ (pD ca. 3) shows a small amount (ca. 10%) of formation of C1′$^{\bullet}$ via 640 nm photoexcitation of the cation radical state ($G^{\bullet+}$:C), whereas spectrum B′ shows very little photoconversion at pD ca. 5.

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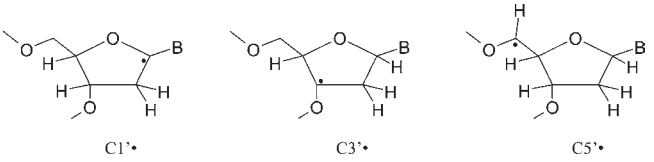
Scheme 2. Structures of the Sugar Radicals Used in This Work[a]



[a] "B" stands for the DNA base.

radical form ($G^{\bullet+}$:C) (spectrum A, black); pD 5, intra-base-pair proton-transferred form ($G(N1-H)^{\bullet}$:$C(+H^{+})^{\bullet}$) (spectrum B, blue); pD 9, a mixture of its deprotonated forms (spectrum C, red) $G(N2-H)^{\bullet}$:C (ca. 60%) and $G(N1-H)^{\bullet}$:C (ca. 40%). At pD 9, the deprotonation takes place from the duplex to the surrounding solvent, i.e., D_2O (see Scheme 1).

3.1.4. d[TATAGCGGCCGCTATA]$_2$. Being an 8-mer, d[TGCGCGCA]$_2$ does not have a complete turn of the double helix. As a result, the proton on N1 in one-electron-oxidized d[TGCGCGCA]$_2$ may have direct access to the solvent. Hence, we have extended the studies regarding the protonation states of one-electron-oxidized guanine to a 16-mer, d[TATAGCGGCCGCTATA]$_2$.

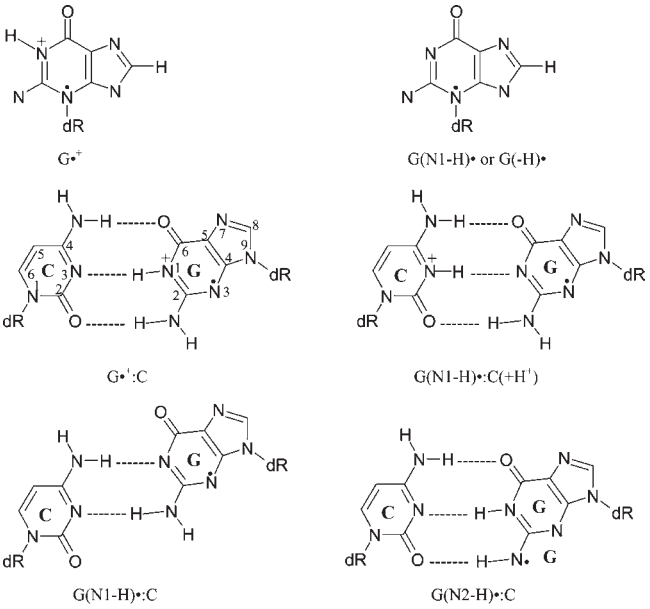
In the left panel of Figure 5 (Figure 5A−C), the ESR spectra of one-electron-oxidized d[TATAGCGGCCGCTATA]$_2$ after thermal annealing to temperatures in the range from 155 to 175 K at different pD values of ca. 3, 5, and 9 in 7.5 M LiCl/D_2O are shown. Following our work regarding characterization of the various protonation states of one-electron-oxidized G in dsDNA oligos[13a,14a] and the studies shown in Figure 4 in this work, we find that at pD ca. 3 the one-electron-oxidized d[TATAGCGGCCGCTATA]$_2$ exists in its cation radical form ($G^{\bullet+}$:C) (spectrum A, black) and at pD 5 the one-electron-oxidized d[TATAGCGGCCGCTATA]$_2$ exists in the intra-base-pair proton-transferred form ($G(N1-H)^{\bullet}$:$C(+H^{+})^{\bullet}$) (spectrum B, blue). However, employing the reported authentic spectra of $dG(N1-H)^{\bullet}$ and $dG(N2-H)^{\bullet}$ derived from one-electron-oxidized dGuo at pD ca. 9[15] and 1-methylguanosine (1-MeGuo) at pD ca. 9,[13a] respectively, as benchmarks, the spectrum of one-electron-oxidized d[TATAGCGGCCGCTATA]$_2$ at pD ca. 9 obtained via annealing at 175 K (spectrum C, red) was found to be an equilibrium mixture of its duplex-to-solvent deprotonated forms $G(N1-H)^{\bullet}$:C (ca. 70%) and $G(N2-H)^{\bullet}$:C (ca. 30%) (Supporting Information Figure S1).

3.2. Photoexcitation Studies. The results of the photoexcitation of the compounds (Figures 2−5) are summarized in Table 1.

Analyses of the data presented in Table 1 result in the following important findings.

3.2.1. pH- and Wavelength-Dependent Formation of the Matrix Radical ($Cl_2^{\bullet-}$) via Photoexcited One-Electron-Oxidized G in Monomer dGuo and in ssDNA and dsDNA Oligomers. In Figures 2 and 3, and in Table 1, we present evidence that only photoexcitation by a 405 nm laser of one-electron-oxidized guanine in its cation radical ($G^{\bullet+}$) state in each of the DNA systems investigated oxidizes the surrounding matrix (homogeneous LiCl solution), resulting in $Cl_2^{\bullet-}$ formation. However, in the case of dsDNA oligomers, $Cl_2^{\bullet-}$ formation is observed via 405 nm photoexcitation of both the cation radical ($G^{\bullet+}$:C) and the intra-base-pair proton-transferred ($G(N1-H)^{\bullet}$:$C(+H^{+})$) forms (see Figures 4 and 5 and Table 1). Moreover, among the

Scheme 3. Structures of the DNA Base Radicals Used in This Work[a]



[a] The numbering scheme for the G:C cation radical is also shown.

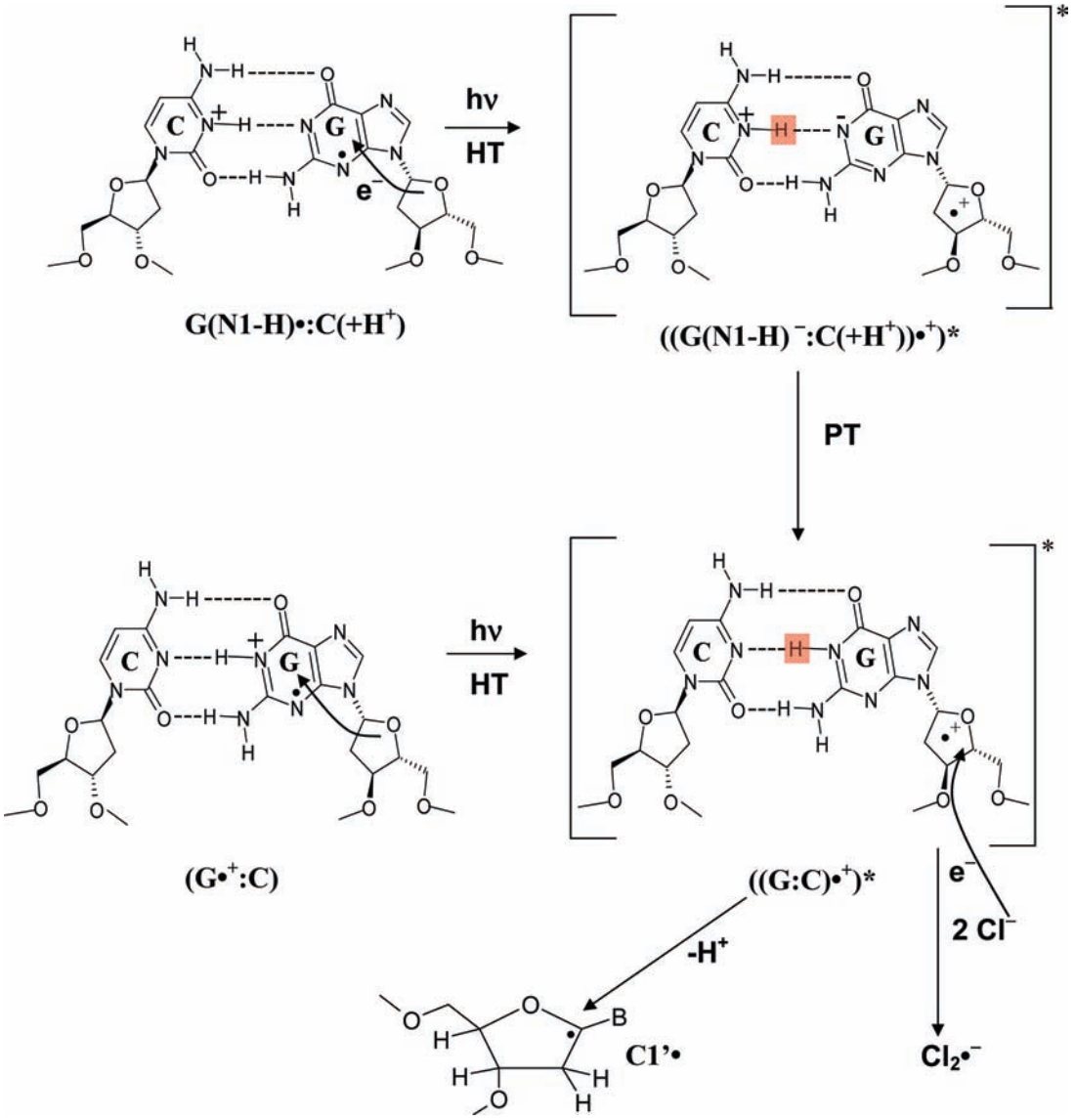
various types of DNA model systems studied in this work, photoexcitation via the 405 nm laser of the $G^{\bullet+}$:C form produces the maximum amount of $Cl_2^{\bullet-}$.

To ascertain whether formation of additional $Cl_2^{\bullet-}$ occurred via photoexcitation of $G^{\bullet+}$ and not as a result of direct photooxidation of the matrix, we performed control experiments. We employed samples of 7.5 M LiCl/D_2O at pD values of ca. 3, 5, and 9 in the presence of $K_2S_2O_8$ handled, γ-irradiated, annealed, and then photoexcited by the 405 nm laser in a manner identical to that of the DNA samples. These results are presented in Supporting Information Figure S2 and show that, upon photoexcitation by 405 nm light, no formation of $Cl_2^{\bullet-}$ occurred. This establishes that the formation of $Cl_2^{\bullet-}$ is caused by photoexcitation of $G^{\bullet+}$ with 405 nm light through hole transfer to the matrix (one-electron oxidation of the matrix).

As discussed in section 3.1, for TGT (ssDNA) and the nucleoside dGuo at pH ca. 5, one-electron-oxidized guanine is an equilibrium mixture (ca. 50% each) of the cation radical ($G^{\bullet+}$) and deprotonated ($G(N1-H)^{\bullet}$) forms. For dGuo and for TGT, owing to the lack of base pairing, the $G(N1-H)^{\bullet}$ form results from deprotonation from $G^{\bullet+}$ to the solvent.[13−15] However, for dsDNA oligomers at pH ca. 5, one-electron-oxidized guanine is in the $G(N1-H)^{\bullet}$:$C(+H^{+})$ form owing to intra-base-pair proton transfer. To explain the $Cl_2^{\bullet-}$ formation via the excited $G(N1-H)^{\bullet}$:$C(+H^{+})$ form in dsDNA oligomers, we propose that, within the lifetime of the excited state of $G(N1-H)^{\bullet}$:$C(+H^{+})$, proton transfer occurring from $C(+H^{+})$ to the N1 atom in $G(N1-H)^{\bullet}$ is required (see Scheme 4). Thus, we propose that, in the excited state, the presence of the guanine N1−H proton is crucial for $Cl_2^{\bullet-}$ formation. We note here that, using photoexcited dsDNA oligomers containing the G:C sequence, the Kohler group[20,21] has shown that the proton transfer occurs within the lifetime of ion-radical exciplexes.

There is no formation of $Cl_2^{\bullet-}$ via 640 nm light in all the compounds studied at all pH values (i.e., in all the protonation states).

Scheme 4. Schematic Representation of the Formation of the Matrix Radical ($Cl_2^{\bullet -}$) and Sugar Radicals via Photoexcited $G^{\bullet +}$:C and G(N1−H)•:C(+H⁺) in DNA[a]



[a] The facile reverse proton transfer from cytosine N3H to guanine N1 via excitation of the G(N1−H)•:C(+H⁺) form is also shown in this scheme.

Further, one-electron-oxidized ds oligomers which are deprotonated to the solvent, i.e., one-electron-oxidized oligomers in the G(N1−H)•:C and G(N2−H)•:C forms, also show no formation of $Cl_2^{\bullet -}$ via photoexcitation at 640 nm as well as at 405 nm.

We conclude that excited states of the one-electron-oxidized G produced by 640 nm light are less oxidative than those created by 405 nm light. Clearly, each wavelength excites a different band in the $G^{\bullet +}$ spectrum,[14] and only the higher energy band results in matrix oxidation.

We note here that, although $Cl_2^{\bullet -}$ is a strong oxidant ($E°(Cl_2^{\bullet -}/2Cl^-)$ = 2.0 V vs the normal hydrogen electrode [NHE] in aqueous solution at room temperature),[19] it cannot oxidize thymidine (Thd) and 2′-deoxycytidine (dCyd) in our system (homogeneous LiCl solution (pH/pD ca. 5) at temperatures near 150 K) via one-electron oxidation. From this observation, and from the reported values of reduction potentials of the DNA base cation radicals,[3,4] we predict that the reduction potential of excited $G^{\bullet +}$ (i.e., $(G^{\bullet +})^*$ for dGuo and TGT and $((G:C)^{\bullet +})^*$ (see Scheme 4) for dsDNA oligomers) is >1.8 V in our system (homogeneous aqueous solution at low temperature).

3.2.2. Sugar Radical Formation in DNA Model Systems (Monomers to dsDNA Oligomers): Influence of the Wavelength, pH, and Length of the Oligomers. Our work shows that the yield of an individual sugar radical and the extent of photoconversion are crucially controlled by the wavelength of the incident light, the pH of the solution, the length and nature of the oligomer. This is illustrated in Table 1 and has led to the following salient points.

3.2.2.1. Effect of the pH. For one-electron-oxidized dGuo, TGT, and the dsDNA oligomers at pD ca. 3, where $G^{\bullet +}$ or $G^{\bullet +}$:C is present, only 30 min of photoexcitation at 405 nm is required for a substantial conversion (ca. 50−90% of the total radicals produced) to the sugar radicals. Even at pD ca. 5, for one-electron-oxidized dGuo and TGT existing in the equilibrium

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Table 1. Formation of $Cl_2^{\bullet-}$ and Sugar Radicals via Photoexcitation of One-Electron-Oxidized Guanine in DNA Systems by 405 and 640 nm Lasers at 143 K and at Different pH Values [a]

compound	λ (nm)	pH	conversion[b] (%) (time, h)	radical yield (%)			
				$Cl_2^{\bullet-}$	C1′•	C3′•	C5′•
dGuo	405	3	90 (1)	15		25	50
		5	90 (1)	10		35	45
		9	20 (0.5)			5	15
	640	3	75 (3.5)		15	30	30
		5	80 (5.5)		15	35	30
		9	− (0.5)				
TGT	405	3	70 (0.5)	30	35		5
		5	70 (0.5)	30	35		5
		9	− (0.5)				
	640	3	30 (3)		30		
		5	30 (5)		30		
$d[TGCGCGCA]_2$	405	3	85 (0.5)	60	20		5
		5	85 (0.5)	30	50		5
		9	− (0.5)				
	640	3	15 (1)		15		
		5	ca. 5 (1)		ca. 5		
$d[TATAGCGGCCGCTATA]_2$	405	3	60 (0.5)	30	25		5
		5	60 (0.5)	20	35		5
		9	− (0.5)				
	640	3	10 (0.5)		10		
		5	ca. 5 (0.5)		ca.5		

[a] Radical yields have a ±10% relative error.[14a] [b] Conversion of one-electron-oxidized G to various sugar radicals and $Cl_2^{\bullet-}$. The total spectral intensities before and after photoexcitation were found to be nearly identical.

mixture of $G^{\bullet+}$ and $G(N1-H)^{\bullet}$, and for dsDNA oligomers existing in the intra-base-pair proton-transferred form $(G(N1-H)^{\bullet}:C(+H^+)^{\bullet})$, we find excellent conversion to sugar radicals via 30 min of photoexcitation at 405 nm. The presence of the N1−H proton in G is crucial for the formation of sugar radicals (see Scheme 4). We already reported formation of sugar radicals via photoexcitation of one-electron-oxidized $d[TGCGCGCA]_2$ using a photo flood lamp in the following order of the pH of the solution: pH ca. 3 > pH ca.5 ≫ pH ca. 9.[13a]

Therefore, this work using lasers of specific wavelengths (405 and 640 nm) and our previous observations using a photoflood lamp[13a] clearly suggest that excitation of $G(N1-H)^{\bullet}:C(+H^+)$ in dsDNA results in reverse proton transfer from the N3 atom of C to the N1 atom of G, thereby leading to excited cation radical $((G:C)^{\bullet+})^*$ formation (see Scheme 4). This proton transfer process must occur within the lifetime of the excited state of this incipient excited cation radical. Subsequent deprotonation from the sugar moiety in the excited cation radical leads to neutral sugar radical formation (see Scheme 4). Such proton transfers in photoexcited dsDNA oligomers containing a G:C sequence have been shown by the Kohler group.[20,21]

We find that, where deprotonation of one-electron-oxidized guanine has occurred to the surrounding solvent, no sugar radicals are formed on photoexcitation. Thus, we conclude that the excitation of $G(N1-H)^{\bullet}$, $G(N1-H)^{\bullet}:C$, and $G(N2-H)^{\bullet}:C$ is ineffective in causing such oxidations either owing to the lifetime or to redox properties of the excited states of these protonation states.

3.2.2.2. Effect of the Wavelength. We find that the total conversion to sugar radicals decreases substantially with increasing wavelength for all DNA systems (i.e., from dGuo to dsDNA oligomers). Note that more extended time periods are needed to photoconvert one-electron-oxidized G in dGuo and in TGT at pD ca. 3 and at pD ca. 5 to the sugar radicals at 640 nm than at 405 nm. For the $G^{\bullet+}:C$ and $G(N1-H)^{\bullet}:C(+H^+)$ forms of the longer dsDNA oligomer $d[TATAGCGGCCGCTATA]_2$, the sugar radical conversion becomes negligible at 640 nm. Moreover, 405 nm photoexcitation in dsDNA oligomers leads to the predominant formation of C1′• along with little formation of C5′•. Sugar radical formation via photoexcitation of $G^{\bullet+}$ in γ-irradiated, hydrated (Γ = 12 D_2O molecules/nucleotide) DNA has been found to be wavelength-dependent.[9] In the 310−480 nm range, formation of C1′• in substantial yields has been observed in dsDNA via photoexcitation of $G^{\bullet+}$ from 77 to 180 K, but little conversion occurs at longer wavelengths.[8,9,11] Therefore, the findings shown in Table 1 bridge our previous findings that a wavelength dependence exists for the formation of sugar radicals in ssDNA oligomers,[11] in $d[TGCGCGA]_2$,[13a] and in highly polymerized DNA.[9] Results obtained by TD-DFT calculations for excited $G^{\bullet+}$ in TpdG and in other dinucleoside phosphates[10] show that base-to-base hole transfer takes place at wavelengths longer than 500 nm, in preference to base-to-sugar hole transfer. Only base-to-sugar hole transfer leads to the formation of sugar radicals (see Scheme 4). Therefore, the substantially lower yields of sugar radicals in dsDNA found at 640 nm are likely explained by the excitation-induced base-to-base hole transfer which competes with the excited base-to-sugar hole transfer.

3.2.2.3. Oligomer Effect. While C3′• is formed via photoexcitation of $G^{\bullet+}$ in dGuo (see the C3′• benchmark spectrum in

Figure1C), the spectra obtained from photoexcited one-electron-oxidized TGT and dsDNA oligomers clearly show that photoexcitation of one-electron-oxidized G in these samples does not produce C3′• and instead forms mainly C1′• and some C5′•.

In the case of dGuo, both the 3′- and 5′-sites have free −OH groups, whereas for G in TGT and in dsDNA oligomers, the 3′- and the 5′-sites have phosphate groups. We have previously provided evidence that the presence of a phosphate group (instead of a hydroxyl group) at the C3′- and C5′- sites deactivates the site to radical formation.[22] This finding was supported by previous theoretical calculations for the relative stability of sugar−phosphate radicals that indicated a destabilization of the resultant radical upon phosphate substitution.[23,24] Thus, in comparison to the predominant C5′• formation and significant C3′• formation via 405 nm photoexcitation of one-electron-oxidized guanine in dGuo, the corresponding 405 nm photoexcitation in TGT and in dsDNA oligomers at both pD ca. 3 and pD ca. 5 shows predominant formation of only C1′• along with no observable C3′• formation and a small yield of C5′•.

3.2.2.4. Length of the dsDNA Oligomer. It is evident from Table 1 that with the increase of the length of the dsDNA oligomer (from an 8-mer to a 16-mer) both the overall yield of the sugar radical, mostly C1′, and the yield of $Cl_2^{\bullet-}$ formed via 405 nm photoexcitation decrease. This decrease in the radical yield for the longer dsDNA oligomer may be a result of the increase in the number of deactivation modes such as base-to-base hole transfer (see section 3.2.2.2).

3.2.2.5. Photostability of C1′• in dGuo. In dGuo samples, 405 nm photoexcitation does not produce observable C1′• (Table 1). C1′• formation is observed only at 640 nm. This suggests that the increased formation of C5′• at 405 nm is the result of either a wavelength dependence on specific radical yields or the photoconversion of already produced C1′• to C5′•. Our preliminary work suggests photoconversion of C1′• to C5′•, and this is being further investigated currently in our laboratory.

4. CONCLUSION

4.1. Mechanism Involved in the Formation of the Matrix Radical ($Cl_2^{\bullet-}$) via Photoexcited One-Electron-Oxidized G. This work provides evidence for two excited-state hole transfer processes induced by excitation of guanine cation radical, $G^{\bullet+}$: (i) transfer to the sugar moiety, leading to neutral sugar radical formation, which has also been shown in previous efforts,[8−12,13a] and (ii) hole transfer to the matrix, forming $Cl_2^{\bullet-}$ (see Scheme 4).

We find that one-electron-oxidized DNA systems in the cation radical form, $G^{\bullet+}$:C, are most active for either sugar radical or $Cl_2^{\bullet-}$ formation, which demonstrates that the presence of the N1−H proton in G is essential to these processes (see Scheme 4). The G(N1−H)•:C(+H^+) form is also found to be active in these processes. The data in Table 1 clearly demonstrate that deprotonation of one-electron-oxidized G in DNA to the surrounding solvent leads to formation of G(N1−H)•:C or G(N2−H)•:C, via which no photooxidation results. Hence, proton transfer from one-electron-oxidized G in DNA to the surrounding solvent prevents hole transfer from one-electron-oxidized G to a sugar or to the matrix. Thus, for the G(N1−H)•:C(+H^+) form, formation of a sugar radical or $Cl_2^{\bullet-}$ is very likely a result of facile reverse protonation in the excited state of G(N1−H)•:C(+H^+) to form $G^{\bullet+}$:C (see Scheme 4).

The formation of sugar radicals and $Cl_2^{\bullet-}$ at 405 nm and the formation of sugar radicals and the complete lack of formation of $Cl_2^{\bullet-}$ at 640 nm (Table 1) are attributed to the fact that each wavelength excites a different band in the $G^{\bullet+}$ spectrum.[14] Only the higher energy 405 nm band results in matrix oxidation. The yield of $Cl_2^{\bullet-}$ on photoexcitation at 405 nm is found to follow the order dsDNA (8-mer) > dsDNA (16-mer) ≈ TGT > dGuo. The lack of formation of either radical on excitation of G(N1−H)•, G(N1−H)•:C, and G(N2−H)•:C suggests their excited states, which are ionic in nature (Scheme 4), have lower redox potentials and likely short lifetimes.

4.2. Relevance of These Studies to the Reported Hole Transfer Processes in DNA. A variety of experimental techniques (e.g., (a) UV−vis photoexcitation of specific sequences of dsDNA oligomers having donors and acceptors at fixed distances at ambient temperatures, (b) ultrafast laser flash photolysis of specific sequences of dsDNA oligomers having donors and acceptors, and (c) ESR spectral studies of irradiated DNA systems at low temperatures) have been used to study the extent and rate of hole transfer processes in DNA.[5−7,13,14,25−31] Our previous work and other reported experimental and theoretical studies have provided evidence for the prototropic equilibria of the hole (i.e., the guanine cation radical) in dsDNA[3−7,23−31] (shown in Scheme 1) and that these reversible proton transfer processes (intra-base-pair proton transfer and duplex-to-solvent deprotonation) crucially control the rate and extent of the hole transfer processes in DNA in the ground state.[5−7,13,14,25−31] The work reported here shows that two excitation events induced hole transfer processes via the ((G:C)•+)* state (Scheme 4), resulting in the sugar radical or matrix radical ($Cl_2^{\bullet-}$), which would clearly terminate such long-range hole transfer processes in DNA.

In the work reported here, annealing of one-electron-oxidized d[TGCGCGCA]$_2$ and d[TATAGCGGCCGCTATA]$_2$ to 175 K resulted in an equilibrium mixture of G(N1−H)•:C and G(N2−H)•:C, with little $G^{\bullet+}$:C expected to be present.[17] Previous work employing γ-irradiated hydrated (Γ = 12 ± 2 H_2O molecules/nucleotide) DNA samples clearly established that formation of •GOH and 8-oxo-$G^{\bullet+}$ occurs via multiple one-electron oxidations via thermally activated hole hopping at ca. ≥200 K.[17] At these elevated temperatures (≥200 K), the prototropic equilibria between various forms of one-electron guanine in DNA (Scheme 1) are established as theory shows the various forms are nearly isoenergetic.[13,14b−14e] Thus, as significant amounts of $G^{\bullet+}$:C become available on annealing above 200 K, nucleophilic addition of water takes place at the C-8 atom in the guanine moiety, leading to the formation of •GOH, which by two subsequent one-electron oxidations forms 8-oxo-$G^{\bullet+}$.[17] Thermally activated hole transfer from one site to another is activated at temperatures above 200 K. Thus, in our system the hole localizes at a guanine site and only moves along the strand by further annealing or photoexcitation.

4.3. Implications of Matrix Radical ($Cl_2^{\bullet-}$) Formation from ((G:C)•+)*. In our system, the high concentration of Cl^- (318 mg of LiCl/mL) facilitated the transfer of holes from ((G:C)•+)* to the matrix via 405 nm photoexcitation. The cell nucleus has a very different environment but is not a dilute aqueous solution owing to the high global concentration of macromolecules (DNA, RNA, proteins, etc.), which ranges from 65 to 220 mg/mL.[32a] The concentration of Cl^-, ca. 4 mM, is of course much lower than in our system.[32] In cellular systems, dsDNA is packaged into nucleosomes with the DNA wrapped around a

histone protein core, which forms the basic unit of the chromatin structure.[32] Consequently, hole transfer from the $((G:C)^{\bullet+})^*$ state in dsDNA to the surrounding nucleoproteins or other oxidizable species would lower the extent of the sugar damage as illustrated in Scheme 4. Our mechanism of formation of the matrix radical ($Cl_2^{\bullet-}$) via $((G:C)^{\bullet+})^*$ may extend to systems in which DNA is closely associated with oxidizable structures. This would, of course, act as a protective mechanism.

■ ASSOCIATED CONTENT

Supporting Information. Figure S1 showing that, employing the $dG(N1-H)^{\bullet}$ spectrum from dGuo and the $G(N2-H)^{\bullet}$ spectrum from 1-MeGuo as benchmarks, the spectrum of one-electron-oxidized $d[TATAGCGGCCGCTATA]_2$ at pD ca. 9 was found to be a mixture of those of its duplex-to-solvent deprotonated forms $G(N1-H)^{\bullet}$:C (ca. 70%) and $G(N2-H)^{\bullet}$:C (ca. 30%) and Figure S2 showing no formation of $Cl_2^{\bullet-}$ in a homogeneous glassy solution of LiCl (7.5 M $LiCL/D_2O$) via direct photoexcitation by a 405 nm laser for 30 min at 143 K. This material is available free of charge via the Internet at http://pubs.acs.org/.

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