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Solvent Exposure Associated with Single Abasic Sites Alters the Base Sequence-Dependence of Oxidation of Guanine in DNA in GG Sequence Contexts

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Abstract

The effect of exposure of guanine in double-stranded oligonucleotides to aqueous solvent on its oxidation by one-electron oxidants was investigated by introducing single synthetic tetrahydrofuran-type abasic site (**Ab**) either adjacent to or opposite tandem GG sequences. The selective oxidation of guanine was initiated by photoexcitation of the aromatic sensitizers riboflavin and a pyrene derivative, and by the relatively small negatively charged carbonate radical anion. The relative rates of oxidation of the 5'-side and 3'-side G in runs of 5'-.GG.. (evaluated by standard hot alkali treatment of the damaged DNA strand followed by high resolution gel electrophoresis of the cleavage fragments) are markedly affected by adjacent abasic sites either on the same or opposite strand. For example, in fully double-stranded DNA or with **Ab** adjacent to the 5'-G, the 5'G/3'G damage ratio is ~4, but is inverted (< 1.0) with **Ab** adjacent to the 3'-G. These striking effects of **Ab** are attributed to the preferential localization of the hole on the most solvent-exposed guanine regardless of the size, charge, or reduction potential of the oxidizing species.

Keywords

DNA; abasic site; oxidative damage; carbonate radical; base sequence effect

Introduction

Macrophages activated in inflammatory tissues produce a wide spectrum of reactive oxygen and nitrogen species that can react with cellular DNA and produce toxic and mutagenic base lesions that have been implicated in the development of diseases associated with the inflammatory response.^[1, 2] Guanine is the most easily oxidizable nucleic acid base^[3] and is thus the primary target of oxidation by reaction with one-electron oxidants.^[4] The formation of oxidatively generated guanine lesions is a base-sequence dependent process since runs of guanines are more easily oxidized than guanines flanked by any of the other DNA

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bases.^[5–8] The enhancement of oxidatively generated guanine damage increases with the number of contiguous guanines according to 5'-G < 5'-GG < 5'-GGG and this phenomenon is correlated with the calculated guanine ionization potentials (IP) in the gas phase,^[5, 9] as well as in the hydrated state in duplex DNA.^[10, 11] The “holes” (guanine radical cations) are delocalized with different probabilities among the different guanines in GG and GGG sequences as shown theoretically^[12–14] and experimentally.^[9, 15, 16] The 5'-G is more easily oxidized and yields greater proportions of hot alkali-labile end-products than the 3'-G in 5'...GG... sequence contexts, while single guanines flanked by bases other than G in double-stranded DNA are much less reactive (for example, references^[5–8, 17]).

Recently, we explored the base sequence-dependent distributions of guanine oxidation products in oligonucleotide duplexes exposed to carbonate radical anions (CO₃•⁻).^[18] The latter are decomposition products of nitrosoperoxycarbonate, an unstable intermediate that is believed to play a role in the oxidative stress associated with inflammation *in vivo*.^[19] We observed that, in the full double-stranded oligonucleotide sequence **F**,

the extent of oxidatively generated damage by the CO₃•⁻ anion radical was indeed ~ 3.5 – 4 times greater at G₃ than at G₄ and the isolated guanines G₂ and G₅; however, the damage observed at the isolated guanines G₁ and G₆ that are close to the ends of the duplexes, were ~ 2.5 greater than at G₃, and ~ 10 times greater than at the guanines G₅ and G₂ that are closer to the center of the duplex **F**.^[18] The strikingly higher yields of oxidatively generated damage at G₁ and G₆ near the ends of the duplexes were attributed to fraying effects that increase the exposure of these guanines to solvent. The distributions of alkali-labile guanine were compared with those generated by the photosensitizers riboflavin (RF)^[20] and the pyrene derivative 7,8,9,10-tetrahydroxytetrahydrobenzo[*a*]pyrene (BPT)^[21] (Scheme) and yielded similar ratios of damage at G₃ and G₄, but the levels were significantly greater than at G₁ and G₆.^[22]

The objectives of this work were to investigate the effects of solvent exposure on the oxidation of guanine in GG sequences in the inner part of 22-mer oligonucleotide duplexes initiated by CO₃•⁻ and BPT•⁺ radicals, as well as by RF*. Partial solvent-exposure was achieved by positioning a tetrahydrofuran abasic site analog (**Ab**) (Scheme) adjacent to a 5'...GG... sequence, or opposite either one or the other of these two guanines in the complementary strand. We find that the additional solvent exposure of G₃ and G₄ with adjacent **Ab** sites can dramatically change the balance of oxidatively generated damage in 5'...G₃G₄ sequence contexts and invert the normally observed G₃/G₄ > 1 ratios. We thus demonstrate that the sequence dependence of oxidatively generated guanine damage in duplex DNA predicted by the Saito model^[5] can be inverted by the selective exposure of guanines in G₃G₄ to the aqueous environment. We speculate that the enhanced solvent exposure and formation of oxidatively generated guanine lesions in the immediate vicinity of abasic sites that are formed in significant numbers in native DNA *in vivo*,^[23] could add to the mutagenic burden of cells under oxidative stress.

RESULTS

Design of oligonucleotide duplexes

The duplexes containing the contiguous guanines ..G₃G₄.. as reporters of the effects of solvent exposure on oxidatively generated damage in the center of the duplexes are shown in Table 1. The two distal single G₁ and G₆ bases near the ends served to minimize fraying effects at the center of the duplexes,^[22] and two proximal single guanines G₂ and G₅ were

used as internal standards for normalizing the DNA damage at G₃ and G₄ in different experiments (Table 1).

The solvent exposure of the G₃G₄ bases was varied by substitution of the flanking T nucleotides with the tetrahydrofuran (1,2-dideoxy- β -ribofuranosyl) residue, **Ab**, that was initially suggested by Millican *et al.* as a stable analog of naturally occurring abasic sites.^[24] In the duplexes **G₄Ab** and **AbG₃** the abasic sites flank G₃ or G₄ on the same strand, while in the duplexes **G₃:Ab** and **G₄:Ab** the abasic sites are positioned in the complementary strand and replace C opposite G₃ or G₄, respectively. The full duplex **F** was used as the control. These duplexes exhibit well defined cooperative melting curves. The average melting temperatures (T_m) calculated from plots of absorbance at 260 nm vs temperature are summarized in Table 1. The destabilization of the DNA duplexes associated with the presence of a single **Ab** site is base-sequence dependent.^[25] The single **Ab** site opposite A bases decreases the value of T_m from 64.0 °C in the control duplex **F**, to 53.5 – 55.0 °C in duplexes **G₄Ab** and **AbG₃**. Substituting the C opposite G by **Ab** is more destabilizing (T_m = 50.9 – 52.0 °C, Table 1) than substituting a T by **Ab** in agreement with literature data.^[25]

Distributions of guanine alkali-labile lesions generated by one-electron oxidants

The photosensitizer RF is an extensively studied type I photosensitizer that selectively initiates the oxidation of guanines in DNA by a one-electron abstraction mechanism.^[9, 26] The photexcited riboflavin molecule RF* is a typical type I photosensitizer, which predominantly oxidizes guanine bases, although one-electron oxidation of adenine and thymine is also possible, but is less efficient.^[27] The type II mechanisms associated with the formation of singlet oxygen are not typical for riboflavin and occur with low efficiency.^[28] The positively charged BPT^{•+} radical cation (generated by a tandem two-photon laser excitation of BPT^[21]) and the negatively charged carbonate radical anion, CO₃^{•-}^[29] also selectively oxidize guanine in double-stranded DNA. Our previous experiments have shown that all these three types of oxidants induce damage predominantly to guanine sites with a greater extent of damage at 5'-G in 5'...GG... sequence contexts^[18, 22] in agreement with the predictions of the Saito model.^[5, 9, 12–14]

The effects of neighboring abasic sites on the extent of damage to guanines at G₃ and G₄ produced by the three different one-electron oxidants generated by photochemical methods^[18, 22] were investigated in the duplexes shown in Table 1. Optimal doses of irradiation or oxidative treatment were chosen to produce a range of quantifiable DNA cleavage fragments in sequencing gels, but under conditions of overall cleavage < 20 – 30%. A single cleavage event per oligonucleotide is observable, according to a Poisson distribution, when less than 30% of the parent duplexes are consumed by strand breaks induced by oxidation followed by hot piperidine treatment.^[30]

Typical cleavage patterns revealed by the standard hot piperidine treatment of duplex **G₄Ab** with the abasic site flanking 3'-T after exposure to the photochemically generated one-electron oxidants are shown in the gel autoradiograph in Figure 1. The strand cleavage is negligible in the unirradiated control samples without (A, lane 1, and B and C, lanes 1 and 2), or with hot piperidine treatment (A, lane 2, and B and C, lanes 3), and without (B and C, lanes 1) or with BPT and riboflavin (B and C lanes 2 and 3). In turn, irradiation of the samples in which the one-electron oxidants are generated, alkali-labile lesions are revealed by the standard hot piperidine treatment. The extent of cleavage occurs in a time-dependent manner (lanes 3–8 in A and B, and lanes 4–9 in B and C) and is observed predominantly at guanine sites as revealed by the alignment of the cleavage bands with the bands in the Maxam-Gilbert lane G (Figure 1).

Typical histograms for RF as the oxidant are shown in Figure 2, and in Supporting Information for the other oxidants. Figure 2 shows that RF* induces damage predominantly at the G-sites. However, a lower level of RF*-induced damage of thymine bases has also been reported since the formation of two alkali-labile lesions, 5,6-dihydroxy-5,6-dihydrothymine and 5-formyluracil has been reported.^[27] In the control duplex **F** without any abasic sites, the extent of the cleavage at G₃ produced by photoexcited RF is markedly greater than that at G₄ (Figure 2A).^[22] However, the substitution of the T bases flanking the G₄ guanine by a single abasic site as in the 5'...TG₃G₄**Ab**T.. sequence context of duplex **G₄Ab**, changes the sequence selectivity of oxidation in the G₃G₄ sequence context since the damage at G₄ is significantly greater than at G₃ (Figure 2B). Introducing a single **Ab** site on the 3'-side of the ...G₃G₄... sequence results in a more efficient damage at G₃ than at G₄ (Figure 2C) than in the control duplex **F** (Figure 2A). In the case of the **G₄:Ab** duplex with the abasic site in the complementary strand opposite **G₄**, the extent of cleavage at G₄ is close to that at G₃ (Figure 2D). However, the positioning of the abasic site in duplex **G₃:Ab** leads to more efficient damage at G₃ than at G₄ (Figure 2E).

In all duplexes, damage at the single inner guanines G₂ and G₅ is quite small under the conditions of irradiation selected and used in these studies (Figures 1 and 2, and Figures S1 and S2 in Supporting Information). The impacts of the abasic site on the oxidation of G₃ and G₄ in the different sequences (Table 1) by the BPT•⁺ and CO₃•⁻ radicals (histograms are shown in Figures S1 and S2 in Supporting Information) are qualitatively similar to those initiated by RF* (Figure 2).

Analysis of relative rates of oxidation of G₃ and G₄

To characterize the impact of abasic sites on the photosensitized oxidation of G₃ and G₄ more accurately, it is necessary to measure the extent of oxidation of each guanine residue as a function of irradiation time, thus focusing specifically on the levels of oxidation produced by the photogenerated oxidizing species. The relative amounts of damage in different duplexes can be estimated by the extent of damage at G₂ and G₅, which are the partially solvent-exposed guanines and are assumed to remain unchanged in all duplexes. The cleavage percentages were calculated from the histograms and normalized relative to averaged damage at the single guanines G₂ and G₅ used as internal standards. Some examples of the effects of irradiation time on the photosensitized oxidation of different guanines by riboflavin are shown in Figure 3 (and for other oxidants in Figures S3 and S4 in Supporting Information); in these plots, the initial levels of oxidation were subtracted from the total levels of oxidation observed at different irradiation times. We then estimated the initial rates of the time-dependent photosensitized oxidation rates (r_3 and r_4) at the G₃ and G₄ sites, respectively, established within the initial few second intervals of irradiation (Figures 3, S3 and S4).

In the case of the **G₄Ab** duplex with the 5'...TG₃G₄**Ab**T...3' sequence context, the cleavage rate at G₄ is greater than at G₃, and in this case both rates at G₄ and G₃ (Figure 3B) are also greater than in the control duplex **F**, in which the cleavage rate at G₄ is less than at G₃ (Figure 3A). The substitution of the 5'-T flanking G₃G₄ by the abasic site in duplex **G₃Ab** also enhances significantly the overall rates of oxidation at both the G₃ and G₄ sites (Figure 3C), but even greater than that in the case of **G₄Ab** (Figure 3B). In the duplex **G₄:Ab** duplex with the abasic site in the complementary strand opposite **G₄**, the rate of cleavage at G₄ is close to that at G₃ (Figure 3D). However, the positioning of the abasic site in duplex **G₃:Ab** leads to a greater rate of damage at G₃ than at G₄ (Figure 3E). The similar trends in the oxidation rates at G₃ and G₄ sites are observed in the case of BPT•⁺ and CO₃•⁻ one-electron oxidants (Figures S3 and S4). The advantage of utilizing the kinetic method is that the slopes are a function of the photosensitizer-induced oxidation of guanine (Figure 3), whereas

straightforward comparisons of peak heights in the histograms (Figure 2) include contributions from background and unspecified prompt mechanisms of DNA damage.

The relative values of r_3 and r_4 obtained for the different oxidants and duplexes are compared in Figure 4. In accord with the Saito model,^[5, 9, 12–14] we find that in the control duplex **F** the values of r_3 are markedly greater than the r_4 values and the r_3/r_4 ratios are ~ 18, 12, and 4.4 in the case of photosensitized oxidation with RF*, BPT•⁺, and CO₃•[−], respectively (Figure 4). The most remarkable effects of abasic sites are observed when the single T flanking the normally weakly reactive G₄ is replaced by the abasic site in the **G₄Ab** duplex. The r_3/r_4 ratios are inverted relative to all of the other duplexes with values of 0.5, 0.7 and 0.5, respectively, when RF*, BPT•⁺, and CO₃•[−], respectively, are used as the oxidants. The inversion of the relative reactivities of G₃ and G₄ is not observed in any of the other three duplexes with abasic sites. When the **Ab** site is positioned in the opposite strand, the largest change in the r_3/r_4 ratios (~ 2.0, 1.4, and 2.2, respectively) is observed in the **G₄:Ab** duplex when the abasic site is positioned opposite G₄ in the complementary strand. In the **AbG₃** and **G₃:Ab** duplexes the differences in the r_3/r_4 ratios are smaller than those observed in the full duplexes **F**.

Oxidation of guanines by the non-selective SO₄•[−] radical

In contrast to the CO₃•[−] anion radical that selectively oxidizes guanine in DNA,^[29] it has been reported that the sulfate radical is capable of oxidizing all four of the natural bases A, G, C and T.^[31] It was therefore of interest to determine the effects of abasic sites in the vicinity of the G₃G₄ sequences on the overall efficiency of oxidation and the distribution of damaged guanine sites. A representative histogram is shown in Figure 5. The overall cleavage patterns are quite similar to those observed in the case of the uncharged bulky sensitizers RF* (Figure 2), the bulky positively-charged BPT•⁺ radicals, and the small negatively-charged CO₃•[−] radicals (Supporting Information). In particular, the inversion of the levels of alkali-labile damage at G₃ and G₄ is observed in the **F** and **G₄Ab** duplexes (Figure 5). These experiments confirm that, even though all four bases are oxidized by SO₄•[−] radicals, the damage is still dominant at guanine sites and shows the same kind of selectivity at GG sites as in the case of the guanine-selective oxidants RF*, BPT•⁺, and CO₃•[−]. This is a consequence of the redistribution of holes among the guanines due to intraduplex hole transfer.^[5, 9]

DISCUSSION

Properties of the tetrahydrofuran abasic site analog

This abasic site is known to thermodynamically destabilize DNA duplexes.^[25] The structural properties of various DNA duplexes containing single **Ab** sites with all bases being present in the complementary strands have been extensively studied by NMR methods.^[32–35] In all of the sequences studied, all base pairs, including those flanking the **Ab** site are characterized by normal Watson-Crick base pairing and a right-handed B-form DNA duplex. While purines opposite **Ab** are stacked within the helix, pyrimidines opposite **Ab** can assume both inserted and extrahelical structures depending on the neighboring base pairs and temperature.^[32, 35] The abasic site is more accessible to solvent than the neighboring base pairs in the duplex and significantly destabilizes the flanking base pairs. The imino protons of base pairs flanking the **Ab** site consequently exhibit a significantly higher rate of exchange with water and broaden more readily than those of other base pairs as the temperature is raised.^[32]

Effects of different oxidants

The photosensitizers of oxidation of guanine utilized in this study, RF^* , $\text{BPT}^{\bullet+}$, $\text{CO}_3^{\bullet-}$, and $\text{SO}_4^{\bullet-}$, are different in size and variable charge. Both RF^* and the pyrene derivative $\text{BPT}^{\bullet+}$ are bulky polynuclear aromatic photosensitizers. Riboflavin was included in these studies because it is the classical photosensitizer used in studies of oxidation of nucleic acids.^[5, 9, 20, 36] The midpoint reduction potential of guanine radicals at pH 7 is $E^\circ[\text{dG}(-\text{H})^\bullet, \text{H}^+/\text{dG}] = 1.29 \text{ V}$ vs. NHE,^[3] while the standard reduction potentials for the carbonate radical is $E^\circ(\text{CO}_3^{\bullet-}/\text{CO}_3^{2-}) = 1.59 \text{ V}$,^[37] and for $\text{BPT}^{\bullet+}$ it is $E^\circ(\text{BPT}^{\bullet+}/\text{BPT}) \sim 1.5 \text{ V}$, all vs. NHE.^[38] Thus, all of these species can oxidize guanine by one-electron transfer mechanisms.^[22] The $\text{SO}_4^{\bullet-}$ radical is a very strong oxidant with $E^\circ = 2.43 \text{ V}$ vs NHE,^[37] and unselectively oxidizes all four natural nucleobases (A, T, G and C).^[31] Even though all four bases are oxidized by $\text{SO}_4^{\bullet-}$ radicals, the damage is still localized on the guanine residues (Figure 5) as it is in the case of RF^* , $\text{BPT}^{\bullet+}$, and $\text{CO}_3^{\bullet-}$ radical oxidants that selectively oxidize only guanine. In the case of $\text{SO}_4^{\bullet-}$ radicals, guanines serve as the “hole” traps for the holes created by $\text{SO}_4^{\bullet-}$ radicals on A, T, or C in double-stranded DNA (Figure 5). Indeed, pulse-radiolysis experiments of Kobayashi and co-workers have shown that the formation of guanine radicals in double-stranded oligonucleotides oxidized by $\text{SO}_4^{\bullet-}$ radicals by hole transfer is extremely fast and that hole localization is completed within a time window of $< 10 \text{ ns}$.^[39, 40]

Impact of abasic sites on the oxidation of guanines and reduction potential of guanine radicals in double-stranded oligonucleotides

There are two different factors that can contribute to the observed effects of neighboring abasic sites on the oxidation of guanines in double-stranded DNA: (i) Accessibility of guanines in DNA to solvent-borne oxidants; abasic sites partially destabilize the local environment^[25] and thus increase the exposure of nucleobases to solvent (e.g.,^[32]). (ii) Enhanced hydration of guanines is known to lower the reduction potentials of guanine radicals,^[10, 11, 17, 41–43] and this may occur to different extents in guanines flanked by or opposite an abasic site. While the first factor is expected to enhance the overall levels of guanine oxidation, the second factor governs the localization of the hole at one of the two guanines and thus the distribution of oxidatively generated damage at the different guanines. Here, we consider these two factors in greater detail.

Accessibility of guanines in DNA to solvent-borne oxidants on rates of electron transfer

The relationships between the first electron abstraction step and the formation of hot alkali-labile guanine oxidation products was considered in detail in our previous publications.^[18, 22] The rate constants, k , of the first electron abstraction step by the $\text{BPT}^{\bullet+}$ radical, depends on the accessibility of the substrate, with values of $k = 1.7 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ for dGMP^[21] and only $4.0 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ in the case of a 22-mer oligonucleotide duplex with two GG sequences in the middle.^[18] Furthermore, k also depends on the guanine sequence, decreasing in the order $\dots\text{GGG}\dots > \dots\text{GG}\dots > \text{G}$ (isolated guanines) with rate constants of $k = 1.5 \times 10^9$, 8.2×10^8 , and $4.0 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$, respectively, in 22-mer oligonucleotide duplexes.^[18] However, in the case of the $\text{CO}_3^{\bullet-}$ radical, solvent accessibility effects are quite small with $k = 6.6 \times 10^7$ ^[29] and $\sim (1.6\text{--}2.0) \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ for dGMP and the 22-mers with either $\dots\text{GGG}\dots$, $\dots\text{GG}\dots$, or isolated $\dots\text{G}\dots$ sequences in the inner portions of these duplexes.^[18] Thus, in contrast to the $\text{BPT}^{\bullet+}$ radicals, in the case of $\text{CO}_3^{\bullet-}$, k is not sensitive to the lowering of the reduction potentials of guanine radicals in $\dots\text{GG}\dots$ and $\dots\text{GGG}\dots$ sequence contexts in double-stranded DNA. This difference has been accounted for in terms of the higher reorganization energy in outer-sphere electron transfer reactions in the case of oxidation of guanine by the carbonate anion radical.^[18]

Distribution of alkali-labile products at G₃ or G₄

The distribution of oxidized guanine residues are rather insensitive to the markedly different oxidants employed in this study and the differences in electron transfer rate constants k . These results clearly show that the distribution of the products is determined after the initial electron transfer step by the subsequent equilibration of holes by hole hopping mechanisms. The equilibration of holes is a key factor which determines the distribution of the end products. Once injected into the duplex that contain G and GG sequences, hole transfer leads to equilibration of holes favoring trapping at 5'-side guanines with the lowest ionization potentials,^[5, 9] thus generating the observed characteristic strand cleavage patterns (Figures 2 – 5, and Supporting Information).

In principle, the flanking abasic sites can also enhance non-covalent binding of intercalators, such as RF^[44] and BPT in DNA^[45] and oligonucleotides.^[46] However, it is shown in Supporting Information that the introduction of abasic sites into double-stranded oligonucleotides does not change the association constant of BPT by more than a factor of two, which will have only a negligible effect under our experimental conditions of 10 μ M oligonucleotide duplexes. With equilibrium association constants of RF and BPT less than $10^3 - 10^4 \text{ M}^{-1}$, the fraction of noncovalently bound RF and BPT are < 20% under the conditions of our experiments (10 μ M DNA). Furthermore, the photoionization of BPT by a two-photon mechanism is a low probability event in BPT-DNA complexes because the singlet excited state, the critical intermediate in the consecutive two-photon absorption process, is strongly quenched by neighboring DNA bases.^[21, 46] At DNA intercalation sites, the lifetimes of the fluorescence-emitting bound BPT singlet excited state is only 1 – 3 ns which is shorter than the width of the actinic laser pulse (8 – 10 ns), thus reducing the yields of BPT^{•+} radical cations.^[21] Previous kinetic laser flash photolysis experiments have shown that the one-electron oxidation of guanines in DNA is indeed mediated by solvent-borne BPT^{•+} radicals;^[18] the pseudo-first-order rate constant of the BPT^{•+} decay depends linearly on the DNA concentration in the range of 0 – 10 μ M, as expected for a diffusive bimolecular mechanism of guanine oxidation by BPT^{•+} radicals.^[18, 22] Analogous considerations apply to the oxidation of guanine in double-stranded DNA by RF* (Supporting Information).

Connections between the one-electron oxidation of guanine and the formation of alkali-labile guanine lesions

Here we consider the different guanine lesions formed and their known susceptibilities to cleavage induced by hot alkali treatment. The oxidation of guanine in DNA by one-electron oxidants triggers a cascade of consecutive chemical reactions that results in the formation of various guanine lesions that are, at a formal level, products of a multi-electron oxidation of guanine.^[47, 48] In the case of BPT^{•+} and RF*, the characteristic products that have been identified and isolated by LC/MS methods are 2,5-diamino-4*H*-imidazolone (Iz) lesions and 8-oxo-7,8-dihydroguanine (8-oxoG).^[49, 50] The contribution of 8-oxoG lesions to the overall extent of guanine oxidation can be estimated as the difference in the yields of cleavage products generated by treatment with the formamidopyrimidine DNA glycosylase (Fpg), and cleavage induced by hot piperidine.^[51–53] The Fpg protein efficiently excises the guanine lesions 8-oxoG, Iz and its hydrolysis product 2,2,4-triamino-5(2*H*)-oxazolone (Oz).^[54–56] In contrast, 8-oxoG is resistant to hot piperidine treatment.^[57, 58] The Iz/Oz lesions are labile under hot alkali conditions and the DNA strands are efficiently cleaved by hot piperidine at the sites of these lesions.^[57] In experiments with BPT^{•+} and RF* the levels of 8-oxoG are ~5 – 20 times lower than those of the Iz lesions^[49, 50] and distributions of strand cleavage assessed either by the Fpg method or the hot alkali treatment alone are expected to be similar.^[7]

The formation of Iz requires the abstraction of four electrons from guanine.^[47, 48] Our laser flash photolysis experiments, using $\text{BPT}^{\bullet+}$ or RF^* as the oxidant, have shown that the major pathway of Iz formation is the combination reaction of guanine radicals with the superoxide radicals.^[50, 59] In these experiments, $\text{O}_2^{\bullet-}$ radicals are formed by the reduction of oxygen by either hydrated electrons derived from photoionization of BPT^[50] or $\text{RF}^{\bullet-}$ radicals^[60] formed via electron transfer from G to RF^* . In these photoinduced reactions, the first electron is abstracted from guanine by the one-electron oxidants $\text{BPT}^{\bullet+}$ or RF^* , and the last three by $\text{O}_2^{\bullet-}$, which is a three-electron oxidant.^[61] In neutral aqueous solutions, the Iz lesions are transformed by spontaneous hydrolysis (~20 h at room temperature) to Oz lesions.^[57] The contribution of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), an important potential product of guanine oxidation,^[4] to the overall piperidine-labile products is unlikely to play a role in our experiments since its formation in aerated aqueous solutions is negligible.^[27]

The oxidation of guanine bases by $\text{CO}_3^{\bullet-}$ radicals also generate alkali-labile lesions.^[29] In this reaction, the formation of the products of four-electron oxidation of guanine (spiroiminodihydantoin, Sp and guanidinohydantoin, Gh) involves the formation of the 8-oxoG intermediates that are product of a two-electron oxidation of guanine.^[62, 63] For example, in oxidation reactions of guanine in single-stranded oligonucleotides by carbonate radical anions, the steady-state levels of 8-oxoG is ~3% or less which is significantly lower than the levels of deeper oxidation products such as Sp.^[62] In double-stranded DNA the major products of oxidation of 8-oxoG oxidation by one-electron oxidants are the Gh lesions,^[64, 65] but formation of small quantities of the Sp lesion have also been detected.^[63] The Gh lesions are typical alkali-labile lesions,^[64, 66] whereas the cleavage of Sp in hot piperidine is incomplete and only ~30%, after one hour at 90°C.^[63] Finally, in our experiments with $\text{CO}_3^{\bullet-}$ radicals, we did not detect any significant differences in the levels or distributions of strand cleavage assessed either by the Fpg method or the hot alkali treatment alone.^[18]

Effects of neighboring abasic sites on reactions of guanine radicals with other reactive radical species

The effects of bases sequence on the rates of end-product formation are expected to be small. In the case of $\text{BPT}^{\bullet+}$, RF^* and $\text{CO}_3^{\bullet-}$, the formation of end-products such as imidazolone and spiroiminodihydantoin occurs by the combination of $\text{G}(-\text{H})^{\bullet}$ radicals with $\text{O}_2^{\bullet-}$ ^[59] or $\text{CO}_3^{\bullet-}$ ^[67] radicals, respectively. The formation of $\text{O}_2^{\bullet-}$ radicals occurs by reaction of O_2 with hydrated electrons that are generated, together with $\text{BPT}^{\bullet+}$ radicals, by the two-photon ionization of BPT^[21], or with $\text{RF}^{\bullet-}$ radicals derived from reaction of RF^* with G.^[20] We have shown previously that the accessibility of $\text{G}(-\text{H})^{\bullet}$ radicals in DNA to small free radicals such as $\text{O}_2^{\bullet-}$ ($4.7 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$)^[59] and $\bullet\text{NO}_2$ ($4.4 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$)^[68] are the same, within experimental error, in the case of single- and double-stranded oligonucleotides. Therefore, the effects of neighboring abasic sites on the accessibility of guanines in DNA to small $\text{CO}_3^{\bullet-}$ and $\text{O}_2^{\bullet-}$ radicals, is unlikely to affect the yields of spiroiminodihydantoin or imidazolone products derived from the combination of $\text{G}(-\text{H})^{\bullet}$ radicals with $\text{CO}_3^{\bullet-}$ ^[67] and $\text{O}_2^{\bullet-}$ ^[59] radicals, respectively.

CONCLUSIONS

In duplexes with single **Ab** sites flanking or opposite guanines, the sequence selectivity of 5'...GG.. oxidation is strongly influenced by the position of the abasic site. These effects are attributed to an enhanced solvent exposure and an increase in the hydration of the guanines closest to the abasic site that decreases the reduction potential of the hydrated guanine radicals. In turn, the lower reduction potential at the exposed guanine radicals flanked by an

Ab site in the 5'-...GG**Ab**... sequence context causes a dramatic inversion of the preferred oxidation of the 5'-G (relative to the 3'-side G) observed in full duplexes and in the absence of abasic sites. The enhanced solvent exposure and formation of oxidatively generated guanine lesion in the immediate vicinity of abasic sites could add to the mutagenic burden of cells under oxidative stress; it has been estimated that mammalian cells undergo up to ~ 10,000 depurination events per day^[23] and their repair, although efficient, may not always occur before a reactive oxygen or nitrogen species interacts with the solvent-exposed guanine.

Experimental Section

Materials

Analytical grade chemicals, HPLC grade organic solvents, and Milli-Q purified (ASTM type I) water were used throughout. The oligonucleotides from Integrated DNA Technologies (Coralville, IA) were purified, and desalted using reversed-phase HPLC. The integrities of the oligonucleotides were confirmed by MALDI-TOF/MS analysis. The damaged strands present in minor quantities in the oligonucleotide samples were cleaved by a standard hot piperidine treatment^[69] and removed by polyacrylamide gel electrophoresis. A pyrene derivative with enhanced water-solubility, 7,8,9,10-tetrahydroxytetrahydrobenzo[a]pyrene was prepared by hydrolysis of racemic *anti*-BPDE (7*r*,8*t*-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, a gift from Dr. S. Amin, Pennsylvania State University, Hershey, PA) and purified by reversed-phase HPLC. A stock solution of BPT (0.5 – 1 mM) in methanol was prepared and small aliquots were added to the oligonucleotide solutions; concentrations of BPT in the sample solutions (~ 10 μ M) were estimated from the molar extinction coefficient of BPT, $\epsilon_{343} = 2.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$.^[70] Riboflavin from Sigma-Aldrich (St. Louis, MO) was used as received; concentrations of riboflavin in the sample solutions (~ 30 μ M) were estimated from $\epsilon_{445} = 1.15 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$.^[71]

Labeling and Annealing of Oligodeoxynucleotides

The oligonucleotide strands (~50 pmol) were labeled at their 5'-termini using OptiKinase (USB, Cleveland, OH) and [γ -³²P]ATP (Perkin Elmer Life and Analytical Sciences, Boston, MA) at 37 °C for 30 min. The labeled samples were purified by polyacrylamide gel electrophoresis; the required bands were cut out, soaked overnight in an elution buffer (0.4 mL, 0.5 M ammonium acetate, 0.01 M magnesium acetate) and isolated by standard ethanol precipitation. The DNA duplexes were prepared by annealing the two strands in phosphate buffer solution (20 mM, pH 7) containing NaCl (0.1 M) at 90 °C for 2 min, and then allowing the samples to cool slowly back to room temperature overnight. The samples used in the photocleavage experiments were prepared by mixing "cold" and radiolabeled strands to obtain the oligonucleotide (~ 10 μ M) solution (~ 50 μ L).

The 260 nm melting profiles of all oligonucleotide duplexes (~2.5 μ M) were determined in phosphate buffer solution (20 mM, pH 7) containing NaCl (100 mM).

Damage to DNA by Photochemically Generated Oxidants

The sample solutions (10 μ L) of duplexes (~ 10 μ M) containing ³²P-5'-end-labeled strands in 2×2 mm square Pyrex capillary tubes (Vitrocom, Inc., Mountain Lakes, NJ) were irradiated for fixed periods of time to initiate strand cleavage.^[18, 22] Depending on the mode of oxidation, the irradiation times were adjusted to maintain the fractions of cleaved oligonucleotide strands below 20%.^[30] Under these conditions, each DNA molecule contains no more than one cleavable site (defined here as a single-hit condition).^[72] To generate SO₄^{•-} (or CO₃^{•-}) radicals, the sample solutions (pH 7.5) containing Na₂S₂O₈ (10 mM) or Na₂S₂O₈ (10 mM) + NaHCO₃ (300mM) were irradiated for fixed periods of time by

a light beam of a 100 W xenon arc lamp reflected at 45° from a dielectric mirror to select the 300–340 nm spectral range for photolysis.^[18] After the irradiation, the reaction mixtures containing persulfate were quenched by the addition of β -mercaptoethanol (0.1 M) or Na₂S₂O₄ (0.1 M) solutions (1 μ L). The BPT radicals cations, BPT^{•+} were produced by two-photon ionization of BPT using 355 nm Nd: Yag laser pulses (~ 25 mJ pulse⁻¹ cm⁻², 10 Hz).^[22, 38, 50] The reaction mixtures containing riboflavin (~ 30 μ M) were excited by 100 mW Xe arc lamp using a dichroic mirror to select the 320 – 360 spectral range. The oxidatively modified DNA was isolated from the photoirradiated samples by standard ethanol precipitation.

Analysis of DNA Damage

The damaged DNA samples were mixed with piperidine (1 M) solution (100 μ L), heated at 90 °C for 30 min, vacuum dried, and the traces of piperidine were removed by repeated lyophilization (2 times). The cleaved oligonucleotide fragments were electrophoresed on a 20% denaturing acrylamide/bisacrylamide (19:1) gel containing urea (7 M) at 3000 V for 2 – 3 h. The vacuum-dried gels were quantitatively assayed using a Storm 840 Phosphorimage System (GE Healthcare). The extent of cleavage was estimated from densitometric traces of the autoradiograms utilizing the Storm 840 software package.

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Figure 1.

Comparisons of strand cleavage patterns in duplex G_4Ab generated by $\text{CO}_3^{\bullet-}$ (A) or $\text{BPT}^{\bullet+}$ (B) radicals, and by photoexcited riboflavin (C) after incubation with hot piperidine and gel electrophoresis (7 M urea, 20% polyacrylamide gel). Lanes G: guanine Maxam–Gilbert sequencing lanes of an unirradiated sequence. (A) Lane 1: unirradiated sequence without piperidine treatment; Lane 2: unirradiated sequence after hot piperidine treatment, Lanes 3 – 8: irradiated sequences (after hot piperidine treatment) irradiated for 2, 5, 10, 20, 40 and 60 s. (B and C) Lanes 1: unirradiated sequence (without piperidine treatment, in the absence of BPT and RF), Lanes 2: unirradiated sequence (without piperidine treatment, in the presence of BPT and RF), Lanes 3: unirradiated sequence (hot piperidine treatment, in the presence of

BPT and RF), Lanes 4–9: irradiated sequence (after hot piperidine treatment, in the presence of BPT and RF) irradiated for 2, 5, 10, 20, 40 and 60s.

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Figure 2.

Representative histograms derived from gel autoradiographs depicting the relative oxidation of guanines by photoexcited riboflavin in duplexes *F* (A), *G₄Ab* (B), *AbG₃* (C), *G₄:Ab* (D), and *G₃:Ab* (E) induced photochemically followed by hot piperidine treatment.

Figure 3.

Kinetics of oxidatively generated damage at G-sites in duplexes *F* (A), *G₄Ab* (B), *AbG₃* (C), *G₄:Ab* (D), and *G₃:Ab* (E) photosensitized by riboflavin. The cleavage percentages were calculated from the histograms of the autoradiographs of denaturing gels and normalized relative to the average damage at the single guanines G₂ and G₅ used as internal standards of oxidizing equivalents in the same duplexes.

Figure 4. Comparisons of initial rates r_3 and r_4 of the photosensitized oxidation of guanines G_3 and G_4 , respectively, in the full duplex F and duplexes with abasic sites G_4Ab , AbG_3 , $G_4:Ab$ and $G_3:Ab$ by photoexcited riboflavin (RF^*), $BPT^{\bullet+}$, and $CO_3^{\bullet-}$ radicals. In each panel, the individual rates (in arbitrary units, a.u.) are expressed relative to the highest rate observed for G_3 in the duplex AbG_3 . The r_4 values for G_4 in the F duplex are the smallest in the case of oxidation by BPT and RF and have been multiplied by a factor of $\times 3$ for better visibility.

Figure 5. Representative histograms derived from gel autoradiographs depicting the relative oxidation of guanines by photochemically generated sulfate radicals in duplexes **F** (A), **G₄Ab** (B), and **AbG₃** (C).

Scheme.

Structures of the tetrahydrofuran abasic site analog (*Ab*, the 1,2-dideoxy-*b*-ribofuranosyl residue); *BPT*, 7,8,9,10-tetrahydroxytetrahydrobenzo[*a*]pyrene; and *RF*, riboflavin).

TABLE 1

Oligo-2'-deoxyribonucleotides

Designation	Oligodeoxyribonucleotide	T_m [a] (°C)
<i>F</i>	5'-TTG ₁ TTG ₂ TTG ₃ G ₄ TTG ₅ TTG ₆ TT 3'-AACAAACAAAC C AAACAAACAA	64.0±0.55
<i>G₄Ab</i>	5'-TTG ₁ TTG ₂ TTG ₃ G ₄ [Ab]TTG ₅ TTG ₆ TT 3'-AACAAACAAAC C A AACAAACAA	53.5±0.5
<i>AbG₃</i>	5'-TTG ₁ TTG ₂ TT[Ab]G ₃ G ₄ TTG ₅ TTG ₆ TT 3'-AACAAACAA A C C AAACAAACAA	55.0±0.5
<i>G₄Ab</i>	5'-TTG ₁ TTG ₂ TTT G ₃ G ₄ TTTG ₅ TTG ₆ TT 3'-AACAAACAAA C [Ab]AAACAAACAA	52.0±0.5
<i>G₃Ab</i>	5'-TTG ₁ TTT G ₂ TTT G ₃ G ₄ TTT G ₅ TTG ₆ TT 3'-AACAAACAAA[Ab]C AAACAAACAA	50.9±0.5

[Ab]: 1,2-dideoxy-D-ribofuranose residue.

[a] Average of two temperature scans from 20 °C to 90 °C with heating rate of 0.4 °C min⁻¹.