Sequence and Stacking Dependence of 8-Oxoguanine Oxidation: Comparison of One-Electron vs Singlet Oxygen Mechanisms

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Abstract: The oxidation of 7,8-dihydro-8-oxoguanine (8-oxoG)-containing oligodeoxynucleotides has been investigated using a variety of oxidants, including one-electron oxidants (Ir(IV), Fe(III), NiCR/$KHSO_5$, and $SO_4^{-\bullet}$) as well as singlet oxygen, generated both photochemically and thermally. The extents of oxidation in single-stranded and duplex oligodeoxynucleotides are compared, confirming theoretical ionization potentials of 8-oxoG in different sequence contexts in duplex DNA. As with guanine, 8-oxoG residues stacked in a duplex with a 3′ neighboring G are more readily oxidized by one-electron oxidants than those stacked next to other bases, although the effect of stacking appears to be less pronounced for 8-oxoG than for G. Regardless of sequence, 8-oxoG is always more easily oxidized than the four natural nucleobases, even in the presence of multiple G sequences. Reactions with singlet molecular oxygen, thought to proceed through a cycloaddition mechanism, show little sequence selectivity and a 7-fold higher reactivity with single-stranded compared to duplex 8-oxoG residues. One-electron oxidants, such as Ir(IV) complexes, showed a more modest 3−4-fold higher reactivity with single-stranded DNA. In contrast, the Schiff base complex $[NiCR]^{2+}$, used in conjunction with a strong oxidant, $KHSO_5$, shows a 2-fold preference for oxidation of duplex vs single-stranded 8-oxoG, perhaps because of the high driving force and the possibility for competing G oxidation to equilibrate to 8-oxoG oxidation via hole transfer. Overall, these results point to subtle mechanistic differences in one-electron oxidation but a major distinction between one-electron and $^1O_2$-mediated oxidation. Furthermore, they suggest an important role for 8-oxoG, not only as a product of oxidative DNA damage but also as a substrate for further oxidation.

Introduction

Oxidative damage to DNA commands interest due to its implications in aging, cancer, and other human diseases.[1−5] Nucleobase damage commonly results in the formation of the 7,8-dihydro-8-oxoguanosine residue (8-oxoG)[6] from guanosine through a variety of mechanisms,[7,8] including attack of a hydroxyl radical at the C-8 position of guanine[9−12] or hydration of the guanine radical cation.[13] Singlet oxygen has also been shown to produce 8-oxoG,[14−16] most likely through a cycloaddition pathway.[17]

Although guanine is the most easily oxidized of the four DNA bases, 8-oxoG has been shown to be an even better substrate for oxidation than any of the natural nucleosides,[18,19] and this second oxidation event has recently become a focal point.[20,21] Various reports place the redox potential for 8-oxoG in the range of 0.58−0.75 V vs NHE,[18,22,23] compared to 1.29 V vs NHE

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for 2′-deoxyguanosine.[24] 8-OxoG oxidation is triggered by a number of reagents and methods: electrochemical oxidation;[25] UV laser photolysis;[26] thermally generated triplet-excited ketones;[27] photoirradiation in the presence of riboflavin,[28] anthraquinone,[29] or fullerenes;[30] γ radiation;[31] enzymatic oxidation using horseradish peroxidase Type VIII,[25] singlet oxygen,[32,33] peroxynitrite,[34,35] iridium (IV),[20] permanganate,[20,36] and osmium-(III); and ruthenium(III) electrocatalysis.[37] Furthermore, aerobic oxidation of 8-oxoG is thought to be responsible for the sensitivity of this lesion to depurination during oligonucleotide synthesis[38] and the modest amount of strand scission observed during piperidine treatment of 8-oxoG-containing oligos.[39]

The electrochemical and enzymatic oxidation products of the nucleoside 8-oxodG (**1**, R = 2′-deoxyribosyl) have been reported by Goyal et al.[25] At pH 7.0, oxidation followed by silylation led to formation of a silylated form of guanidinohydantoin-2′-deoxyribonucleoside (**3**). Similarly, we found that one-electron oxidation of 8-oxoG-containing oligodeoxynucleotides at 25 °C led to guanidinohydantoin, assigned as the major, piperidine-labile product on the basis of ESI-MS (Scheme 1).[21] A minor product, thought to be azaspirodihydantoin,[40] is also formed.[41−43]

Photooxidation studies of 8-oxoguanosine derivatives with singlet molecular oxygen ($^1\Delta_g$) at low temperature in organic solvent provided evidence for initial formation of a dioxetane intermediate that can lead to a variety of products, depending upon the reaction conditions (Scheme 1).[27,32,33,44,45] These products include cyanuric acid **5** as a major product in studies with 8-oxodG,[33] as well as five- and seven-membered ring heterocycles **4** and **6** as major products of $^1O_2$ oxidation of silylated 8-oxoguanosine in organic solvent.[32] Another pathway has been proposed to lead to imidazolone **7** and oxazolone **8** (or its ring-opened isomer **9**) as the final products of 8-oxoG oxidation using $^1O_2$.[27,33,46]

Scheme 1

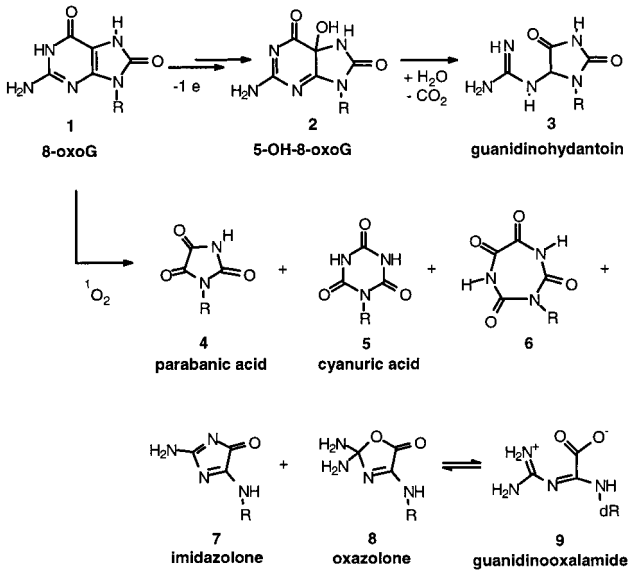

Table 1. Calculated Ionization Potentials for G and 8-oxoG (O) in Various Sequence Contexts for Duplex DNA Models

	IP (eV) (Prat et al.)[a]	IP (eV) (Saito et al.)[b]
G	7.31	7.75
*G*T		7.69
*G*C		7.68
*G*A		7.51
*G*G	6.64	7.28
*G*GG		7.07
O	6.93	
G*O*	6.51	
*O*G	6.38	

[a] B3LYP/6-31*, ref 53. [b] HF/6-31G*, ref 50.

As early as 1985, it was reported that the ease of oxidation of guanine residues by oxidants such as ionizing radiation was sensitive to sequence.[47] Specifically, guanine residues located 5′ to a purine, especially guanine, were more reactive than those located 5′ to a pyrimidine when stacked in a regular B helix.[48] This was further illustrated experimentally by Saito et al,[49] and calculations by this group showed the trend in ionization potentials to be 5′-GGG-3′ < 5′-GG-3′ < 5′-GA-3′ < 5′-GT-3′ ∼ 5′-GC-3′ < G (Table 1).[50] These guanine repeat sequences, especially 5′-GG-3′, have been used as traps to examine electron hole transfer in duplex DNA.[51,52]

Recent calculations by Prat et al. suggest that the redox potential of 8-oxoG may be similarly influenced by neighboring bases.[53] Ionization potentials obtained from ab initio calculations

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of base-paired 5′-OG- 3′ vs 5′-GO-3′ sequences (O = 8-oxoguanosine) indicated that a 3′-guanosine neighbor will facilitate further oxidation of 8-oxoG (Table 1). Some data consistent with this idea already exist for single-stranded oligodeoxynucleotides,[36,54−56] although the effects of π-stacking are unknown for single-stranded oligomers.[57] Thus, the sequence effects on 8-oxoG oxidation remain to be confirmed by experiment for duplex DNA under "single-hit" conditions. Here we present a systematic study of one-electron (Ir^{IV}, Fe^{III}, NiCR/$KHSO_5$, and $SO_4^{-\bullet}$) vs singlet oxygen (generated both photochemically and thermally) oxidation of double-stranded vs single-stranded oligodeoxynucleotides containing 8-oxoG in a variety of sequence contexts.

Experimental Section

Materials. Reagents were purchased from the following sources: Na_2IrCl_6 and Na_2IrBr_6 from Alfa Aesar, $K_3Fe(CN)_6$ from Spectrum, $K_2S_2O_8$ from Aldrich, $KHSO_5$ from Sigma, Rose Bengal and piperidine from Acros, 8-oxodG phosphoramidite from Glen Research, T4 polynucleotide kinase from New England Biolabs, and [γ-^{32}P]ATP from Amersham Pharmacia. NiCR, (2,12-dimethyl-3,7,11,17-tetraazabicyclo-[11.3.1]heptadeca-1(17)2,11,13,15-pentaenato)nickel(II) perchlorate, was synthesized as previously described.[58] Oligodeoxynucleotides were synthesized with an Applied Biosystems synthesizer (ABI 392B) using phosphoramidites from Perkin-Elmer and incorporating 0.25 M β-mercaptoethanol into the final, manual deprotection step for oligomers containing 8-oxoG.[59] Oligos were 5′-end-labeled using T4 polynucleotide kinase and [γ-^{32}P]ATP. Radioactivity was quantified using a Beckman LS6500 scintillation counter. All aqueous solutions were prepared with nuclease-free water (Promega) and reagents of the highest commercial quality.

Oxidation Reactions. Oxidation reactions were carried out on five DNA oligomers using the parent sequence: 5′-d(TCATGGGTCGTCGGTATA)-3′ (**10**), along with sequences in which certain guanine residues were replaced with 8-oxoG: 5′-d(TCATGGGTCOTCGGTATA)-3′ (**11**); 5′-d(TCATGGGTCGTCOGTATA)-3′ (**12**); 5′-d(TCATGGGTCGTCGOTATA)-3′ (**13**); and 5′-d(TCATGOTCGTCOGTATA)-3′ (**12**) (where O = 8-oxoG). Sequence **15**, 5′-d(TATACCGACGACCCATGA)-3′, was the complementary sequence used in all the double-stranded reactions involving sequences **10**−**13**, while sequence **16**, 5′-d(TATACCGACGACCATGA)-3′, was used as the complementary strand in duplex reactions with sequence **14**. Each reaction was prepared by combining 5 μL of 1 M NaCl and 100 mM NaP_i (pH 7.0), 5 μL of 30 μM DNA (**10**−**14**), 5 μL of 5′-end-labeled DNA (**10**−**14**) (9 nCi), 5 μL of 33 μM (**15** or **16**) (for double-stranded reactions only), and H_2O to a final volume of 48 μL. Double-stranded reactions were annealed by heating in a 90 °C water bath for 1 min. The water bath was then turned off, and the samples were allowed to cool slowly to 35 °C (~3 h).

The metal-catalyzed oxidation reactions were initiated by addition of 2 μL of a stock solution of one of the following metal complexes: Na_2IrCl_6, Na_2IrBr_6, or $K_3Fe(CN)_6$ to a final concentration of 10−20 μM for the Ir(IV) complexes and 400 μM for the Fe(III) complex. The reactions were maintained at 25 °C for 1 h in a water bath and then quenched by addition of 2 μL of 250 mM EDTA and 50 mM HEPES (pH 7.0). For reactions with NiCR, $KHSO_5$ (10−60 μM) was added 10 min after addition of the metal complex (3 μM) to initiate oxidation, and quenching was carried out after 30 min.

The sulfate radical reactions involved addition of 2 μL of 12.5 mM $K_2S_2O_8$ (final concentration 0.5 mM), followed by illumination with a 254-nm (6 W) UV lamp (UVP) at a distance of 10 cm for 7 min[60] and then quenching as above. All reactions were dialyzed overnight with 3500 MWCO dialysis membrane against nanopure water.

The singlet oxygen reactions were performed by irradiation of the reaction mixture with a 300-W tungsten lamp at a distance of 20 cm in the presence of 20−50 μM Rose Bengal for 10−30 min at 12 °C. To completely remove the Rose Bengal from the reaction mixture, each reaction was passed through a G-25 gel filtration microspin column (Pharmacia) and then dialyzed as described above. Singlet oxygen was also thermally generated from $NDPO_2$ (disodium 3,3′-(1,4-naphthylidine)dipropionate endoperoxide).[61,62] Although the results were qualitatively similar, background reactions were a serious problem in this case due to incomplete removal of the oxidizing species.

After dialysis, each reaction was lyophilized to dryness and treated with 60 μL of 0.2 M piperidine (freshly prepared) at 90 °C for 30 min to effect strand scission at oxidized sites.[5] The piperidine was removed by lyophilization, and each reaction was dissolved in 4 μL of 6 M urea loading buffer and loaded onto a 20% polyacrylamide gel. The gel was fixed and dried and then exposed to a Molecular Dynamics phosphorimaging screen overnight. Individual bands were quantified as a percentage of the total reaction, after correction for background, using ImageQuaNT software from Molecular Dynamics. The reported values are averages of at least three runs, and errors are estimated to be <10%.

(^{32}P)-5′ TCAT**GGG**TC**G**TC**GG**TATA 3′ (10)
3′ AGTACCCAGCAGCCATAT 5′ (15)

(^{32}P)-5′ TCAT**GGG**TC**O**TC**GG**TATA 3′ (11)
3′ AGTACCCAGCAGCCATAT 5′ (15)

(^{32}P)-5′ TCAT**GGG**TC**G**TC**OG**TATA 3′ (12)
3′ AGTACCCAGCAGCCATAT 5′ (15)

(^{32}P)-5′ TCAT**GGG**TC**G**TC**GO**TATA 3′ (13)
3′ AGTACCCAGCAGCCATAT 5′ (15)

(^{32}P)-5′ TCAT**GO**TCGTC**OG**TATA 3′ (14)
3′ AGTACCAGCAGCCATAT 5′ (16)

Figure 1. Sequences of synthetic oligodeoxynucleotides used in this work; O = 8-oxoG. Only sequences **10**−**14** were 5′-end-labeled with [^{32}P]phosphate.

Results and Discussion

Oligodeoxynucleotide Design, Synthesis, and Characterization. The sequences of the oligomer substrates used in this study were designed such that the reactivity of G and G repeat sequences vs 8-oxoG could be compared within one (intramolecular) and between separate (intermolecular) oligomers (Figure 1). To challenge 8-oxoG residues (O) with G repeat sequences, a GGG segment was placed on the 5′ side of the O site in oligos **11**−**13** such that the use of 5′-end-labeling would overestimate, rather than underestimate, the reactivity of the GGG, in the event that any reactions took place more than once per strand. This problem arises because a second oxidation event, leading to cleavage occurring on the 5′ side of the first, will result in loss of information of the first oxidation when 5′ end-labeling is employed.[63] Oligomers **12** and **13** were designed to test the effects of stacking of an adjacent guanosine residue on either the 3′ or 5′ side of O, respectively. Oligomer **14** competes these two sequence motifs intramolecularly.

The synthesis of 8-oxoG-containing oligomers followed the Glen Research protocol, in which β-mercaptoethanol was

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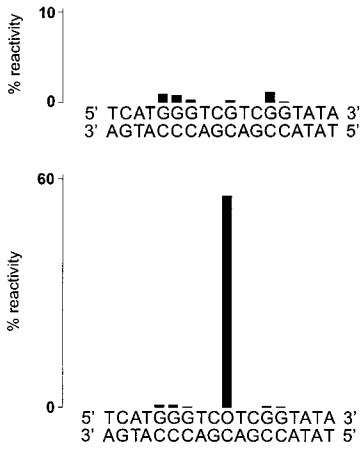

Figure 2. Percent reactivity, corrected for background, of G and 8-oxoG sites in duplex oligodeoxynucleotides analyzed by PAGE. Reactions were carried out with 3 μM NiCR and either 60 μM $KHSO_5$ for oligo **10·15** (top panel) or 40 μM $KHSO_5$ for oligo **11·15** (bottom panel), followed by piperidine treatment to effect strand scission. For duplex reactions, sufficiently high concentrations of oxidant were chosen in order to visualize G oxidation for comparison, despite the fact that this led to >30% reactivity overall.

incorporated into the final, manual deprotection stage of solid-phase synthesis in order to avoid overoxidation of the O residues.[38,59] The identity and purity of the oligomers containing 8-oxoG were confirmed by negative ion electrospray MS (see Supporting Information). In addition, each oligomer was sequenced with dimethyl sulfate, and the location of 8-oxoG was confirmed by reaction with Na_2IrCl_6.[20]

Reactivity of 8-oxoG vs G Multiples. In previous work, we found that square-planar nickel(II) complexes in the presence of a peracid, $KHSO_5$, oxidize guanine residues via a one-electron mechanism.[64] Indeed, a large number of transition metal-mediated and photochemical processes lead to this same result. It is now well established that guanine residues in repeat sequences are more reactive than guanine residues followed by non-guanine residues in duplex DNA, and an example of this phenomenon is shown in Figure 2 (top). Computed ionization potentials for double-stranded 5′-GG-3′ and 5′-GGG-3′ sequences were reported by Saito et al. as 7.28 and 7.07 eV, respectively, both of which are lower than that for a guanine residue with a non-guanine 3′ neighbor (≥7.51 eV).[49] What was unclear at the outset was whether the GG stacking effect would be large enough to allow a G repeat sequence (GG or GGG) to compete in an oxidation reaction with the intrinsically lower oxidation potential of 8-oxoG, as suggested by computational results of Prat et al.[53]

In the experiment, comparison of the reactivity of guanine repeat sequences such as 5′-GG-3′ and 5′-GGG-3′ with 8-oxoG in double-stranded DNA toward NiCR/$KHSO_5$ showed that 8-oxoG is much more easily oxidized than any stacked guanine repeat sequence. Duplex oligonucleotide **11·15** was subjected to oxidation by NiCR/$KHSO_5$, and the percent reactivity of each reactive site was quantified (Figure 2, bottom). 8-OxoG oxidation accounted for 55.1% of the total reactivity, while the combined reactivities of all the guanine residues in the 5′-GG-3′ and 5′-GGG-3′ sequences were 0.5% and 1.5%, respectively. While a total reactivity >50% could lead to multiple events on the same strand, these results still suggest that 8-oxoG is minimally 30 times more reactive than a 5′-GGG-3′ repeat sequence in duplex DNA. Similar results were obtained using other oxidation systems, such as sulfate radical, produced by photolysis of potassium persulfate, and singlet oxygen, generated by photoactivation of Rose Bengal. Furthermore, concurrent studies by Ropp and Thorp also show that 8-oxoG is more readily oxidized than a GGG stacked sequence using Os^{III} electrocatalysis.[37]

Table 2. Reactivity Ratios of Single- vs Double-Stranded (ss:ds) 8-oxoG Residues Using **11** vs **11·15** for Various Oxidants

oxidant	$E_{1/2}$ (V vs NHE)	mechanism	ss:ds
$Ni^{III}CR\ SO_4^{-\bullet}$	>1.3	one-electron	0.5
$IrCl_6^{2-}$	0.90	one-electron	2.0
$IrBr_6^{2-}$	0.82	one-electron	3.4
$Fe(CN)_6^{3-}$	0.42	one-electron	1.3
$SO_4^{-\bullet}$	>2	one-electron and addition	3
$^1O_2$		cycloaddition	7.1

Calculations by Prat et al. provided information regarding the theoretical ionization potentials of G and 8-oxoG (Table 1).[53] The present experiments are in general agreement with the computational and other experimental data[20,31,36,44] that showed 8-oxoG to be more easily oxidized than G. However, they do not agree with the absolute ranking of GG vs 8-oxoG, in which computations suggested that a 5′-GG-3′ sequence would be more easily oxidized than 8-oxoG (Table 1). Apparently, the stacking influence on lowering the oxidation potential of G is overestimated in the calculations.

A recent report showed that $KMnO_4$-mediated oxidation of 8-oxoguanosine residues in single-stranded and duplex DNA could mediate damage to neighboring bases.[36] Unlike $KMnO_4$, we found no special reactivity of adjacent bases when one-electron oxidants or $^1O_2$ were used under the conditions described herein. Permanganate reacts by multiple mechanisms, including dihydroxylation of thymines,[65] and this may account for its different behavior compared to that of clean, one-electron oxidants such as Ir^{IV}.

Reactivity of 8-oxoG in Single-Stranded vs Duplex DNA. The reactivity of guanine toward one-electron oxidation to form guanine radical cation has been observed to be sequence dependent in duplex DNA but non-sequence dependent in single-stranded DNA.[64] To examine whether 8-oxoG exhibits a similar reactivity pattern, single- and double-stranded oligodeoxynucleotide **11** (vs **11·15**) containing a single 8-oxoG was subjected to oxidation by a number of oxidation systems, including NiCR/$KHSO_5$, $IrCl_6^{2-}$, $IrBr_6^{2-}$, $Fe(CN)_6^{3-}$, $SO_4^{-\bullet}$, and $^1O_2$. NiCR/$KHSO_5$, $IrCl_6^{2-}$, $IrBr_6^{2-}$, and $Fe(CN)_6^{3-}$ act as one-electron oxidants with redox potentials of ≥1.2,[58] 0.90,[20] 0.82,[20] and 0.42 V (vs NHE),[66] respectively. The ratios of reactivity of 8-oxoG in single- vs double-stranded contexts (ss:ds) are shown in Table 2.

Curiously, NiCR/$KHSO_5$ showed more reactivity toward 8-oxoG in duplex DNA, while $IrCl_6^{2-}$, $IrBr_6^{2-}$, and $Fe(CN)_6^{3-}$ were more reactive in single-stranded DNA. The heterocyclic bases are more accessible in single-stranded DNA, facilitating an inner-sphere oxidation which might be required for weaker one-electron oxidants. However, a stronger oxidant such as NiCR/$KHSO_5$ may more easily remove an electron from DNA, whether it be single-stranded or duplex. Importantly, NiCR/$KHSO_5$ is also a powerful enough oxidant to oxidize guanine residues in addition to 8-oxoG. Since there are seven G's in duplex **11·15**, five of them being in the same strand as the 8-oxoG residue, much of the additional reactivity of 8-oxoG seen for NiCR/$KHSO_5$ could be due to initial formation of a

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Table 3. Intermolecular Sequence Dependence of 8-oxoG (O) Oxidation in Double-Stranded (ds) DNA

oxidant	ds (5′-*O*G-3′)[a]	ds (5′-G*O*-3′)[b]	ds (*O*)[c]
$NiCR/KHSO_5$	10.9	10.2	12.2
$IrCl_6^{2-}$	30.4	12.7	9.2
$IrBr_6^{2-}$	10.9	4.5	3.1
$Fe(CN)_6^{3-}$	15.8	15.0	14.4
$SO_4^{-\bullet}$	6.7	5.9	5.2
$^1O_2$	10.9	9.0	6.9

[a−c] Data were calculated from the extent of 8-oxoG oxidation, visualized as piperidine-sensitive strand scission using oligomers **12·15**,[a] **13·15**,[b] or **11·15**.[c]

guanine radical cation, followed by hole migration in the duplex to the more stable radical cation at the 8-oxoG residue.[29] Interestingly, this behavior of the $NiCR/KHSO_5$ system is the opposite of what was observed with guanine oxidation; in that case, G's exposed in single-stranded regions were at least 10-fold more reactive than those stacked in a duplex. For guanine, this was attributed to the ability of the N7 lone pair to bind to an intermediate nickel(III) species, thereby facilitating oxidation of that base.[67] 8-OxoG is less likely to bind metals since its predominant tautomer has a proton on N7, and the lone pair is presumably part of the π system of the heterocycle. Thus, 8-oxoG oxidation appears to benefit from the presence of other guanines in the sequence when the oxidizing agent is one with a high driving force, such as $NiCR/KHSO_5$. These guanines may act as antennae to direct additional oxidative damage to 8-oxoG via the helix.

Sulfate radical can act as a one-electron oxidant, but it can also oxidize DNA nucleobases through an addition/elimination mechanism, similar to $HO^{\bullet}$.[7] $SO_4^{-\bullet}$ is more reactive toward 8-oxoG in single-stranded DNA, possibly indicating that the mechanism of oxidation is predominantly addition, and therefore nucleobase accessibility is an important factor. Similarly, singlet oxygen showed >7 times more reactivity in single-stranded DNA compared to that in duplex DNA. Singlet oxygen clearly reacts with 8-oxoG via a different mechanism, likely through a cycloaddition process with the 4,5-double bond, which is again more accessible in single-stranded DNA.

Reactivity of 8-oxoG in Different Sequence Contexts. Ab initio calculations of 5′-OG-3′ and 5′-GO-3′ in a stacked duplex conformation showed the ionization potential of the 5′-OG-3′ sequence to be slightly lower than that of the 5′-GO-3′ sequence.[53] In essence, the electron-rich lone pair on G-N7 can contribute electron density toward the heterocycle on its 5′ side because of its position in the helix. By examination of the double-stranded reactivity of oligos **12·15** and **13·15** toward the oxidant systems previously described, it can be seen that an 8-oxoG located 5′ to a guanine residue is more reactive than when it is 3′ to a guanine residue. Table 3 shows the reactivity of 8-oxoG and the adjacent guanine residue in the 5′-OG-3′ (**12·15**) and 5′-GO-3′ (**13·15**) sequences, in addition to the reactivity of 8-oxoG with non-guanine neighbors (**11·15**) for a series of oxidants. The data for $NiCR/KHSO_5$ and $^1O_2$ oxidations are also shown graphically in Figures 3 and 4, respectively. For one-electron oxidants, the 8-oxoG located 5′ to a guanine was more reactive than 8-oxoG located 3′ to a guanine residue when the bases were stacked in a duplex (compare boxed regions of Figure 3), whereas the ratio of O:G reactivity in single-stranded oligos was independent of sequence (Figure 3). Additionally, in most cases, 8-oxoG with non-guanine neighbors was less reactive than 8-oxoG with a 5′ or 3′ neighboring guanine residue.

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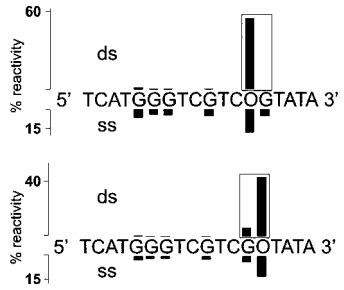

Figure 3. Sequence dependence of one-electron oxidation of 5′-OG-3′ vs 5′-GO-3′ in double-stranded (ds) vs single-stranded (ss) oligodeoxynucleotides. Reactions were carried out using 3 μM NiCR and 40 μM $KHSO_5$, followed by piperidine treatment, for oligomers **12** (top panel) and **13** (bottom panel). A sufficiently high concentration of oxidant was chosen to be able to compare ss and ds reactions under the same conditions, despite the fact that this led to >30% reactivity overall. Boxed areas highlight the sequence-dependent nature of the one-electron oxidation.

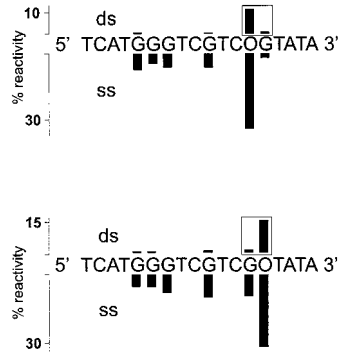

Figure 4. Sequence dependence of $^1O_2$-mediated oxidation of 5′-OG-3′ vs 5′-GO-3′ in double-stranded (ds) vs single-stranded (ss) oligodeoxynucleotides. Reactions were carried out using 50 μM Rose Bengal, followed by piperidine treatment, for oligomers **12** (top panel) and **13** (bottom panel). Boxed areas highlight the observation that $^1O_2$-mediated oxidation is sequence independent in duplex DNA, yielding a constant ratio of G:O oxidation.

Table 4. Intramolecular Sequence Dependence of 8-oxoG (O) Oxidation in Duplex **14·16**

oxidant	ds (5′-*O*G-3′)	ds (5′-G*O*-3′)
$NiCR/KHSO_5$	6.0	4.3
$IrCl_6^{2-}$	17.4	4.7
$IrBr_6^{2-}$	8.4	3.5
$Fe(CN)_6^{3-}$	5.3	7.9
$SO_4^{-\bullet}$	2.6	5.8
$^1O_2$	6.7	5.0

In contrast, $^1O_2$ showed no sequence preference in oxidation of 8-oxoG (compare boxed regions of Figure 4), consistent with the lack of a radical cation intermediate in this mechanism.

The studies described above compared sequence dependence in an intermolecular sense. To verify the influence of neighboring bases in a duplex helix on 8-oxoG oxidation, we synthesized one oligomer containing both the 5′-OG-3′ and 5′-GO-3′ sequences. Care was taken to place the less reactive 5′-GO-3′ segment nearer the 5′ end, such that its reactivity would not be underestimated in the event of multiple oxidation events occurring on the same strand that is 5′-end-labeled. This intramolecular competition study (Table 4) of 8-oxoG with a 5′ or 3′ guanine neighboring base showed the difference in reactivity of 8-oxoG in each sequence to be similar to that in the intermolecular study (Table 3), where $NiCR/KHSO_5$, $IrCl_6^{2-}$, $IrBr_6^{2-}$, and singlet oxygen were used as the oxidant. 8-oxoG with a 3′ neighboring guanine residue was 1.4, 3.7, 2.4, and

1.3 times more reactive than the 8-oxoG with a 5′-neighboring guanine, respectively (Table 4). Interestingly, in this study, $Fe(CN)_6^{3-}$ and $SO_4^{-\bullet}$ demonstrated more reactivity toward 8-oxoG with a 5′ guanine neighbor than 8-oxoG with a 3′ guanine neighbor. This effect was reproducible through >10 trials; moreover, the total extent of reaction was always less than 30% for these reagents, so that multiple hit errors were minimal. Thus, the reason $Fe(CN)_6^{3-}$ and $SO_4^{-\bullet}$ do not fit the pattern of other one-electron oxidants is not immediately clear. Perhaps these results imply subtle differences in reaction mechanism when oxidants of relatively low potential, such as $Fe(CN)_6^{3-}$, or oxidants that react by multiple mechanisms ($SO_4^{-\bullet}$) are employed.

Conclusions

It has already been well established that stacking influences in the B helix lower the oxidation potential of G repeat sequences compared to those of 5′-GA-3′, 5′-GC-3′, or 5′-GT-3′. However, this effect is not sufficiently large to allow a 5′-GGG-3′ duplex sequence to compete significantly with 8-oxoG as a site of oxidation. In all cases studied here, 8-oxoG was the preferred site of oxidation, independent of the mechanism of oxidation. The O:G preference was sometimes as high as 30-fold.

Due to increased accessibility, 8-oxoG is more easily oxidized in single-stranded DNA vs duplex DNA, with $NiCR/KHSO_5$ being the only exception. This is likely due to its high redox potential and its ability to oxidize G residues, leading to additional 8-oxoG oxidation after hole migration. One-electron oxidants typically show a preference for 5′-G oxidation in duplex DNA strands containing multiple G's. Among these oxidants, subtle differences were observed and attributed to (a) the driving force of oxidation and (b) the possibility of other mechanisms, such as addition/elimination, as in the case of sulfate radical. For singlet oxygen, the discrepancy between single- and double-stranded reactivity was even more pronounced, and singlet oxygen strongly prefers to undergo cycloaddition with the accessible 8-oxoG residues found in single-stranded structures.

A principal goal of this research was to determine whether 8-oxoguanosine residues stacked in a B helix were subject to the same electronic effects due to a 3′ guanosine neighbor. The experimental data reported here are in general agreement with the theoretical calculations performed by Prat et al.[53] In duplex DNA, 8-oxoG was oxidized more easily, in most cases, when followed by a guanine residue as opposed to having a 5′ guanosine neighbor. The results in Figure 2 demonstrated that a guanine followed by another guanine is 3−4 times more reactive that a guanine which is followed by a non-guanine residue. This same effect was observed with 8-oxoG oxidation (Figure 3) but generally to a lesser extent, the preference being typically about 2-fold. Additionally, both sequences were generally more easily oxidized than 8-oxoG flanked by non-guanine residues. However, the experimental data were not consistent with the absolute ranking of GG vs 8-oxoG; as noted above, 8-oxoG was always the prevalent site of reaction, even when challenged with a GGG sequence in the same strand.

Taken together, these data may help predict sites of oxidative damage in DNA on the basis of sequence and stacking parameters. Since 8-oxoG is the most common oxidative lesion in DNA, and because a second oxidative event, if occurring by a one-electron mechanism, may trigger hole migration over hundreds of angstroms in duplex DNA,[68] it is clear that second oxidation events at 8-oxoG will be important in understanding DNA damage.

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Supporting Information Available: ESI-MS data, characterizing 8-oxoG-containing oligomers, and representative gel electrophoretic data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA991929Q

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