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Formation of 8-oxo-7,8-dihydroguanine-radicals in γ-irradiated DNA by multiple one-electron oxidations

Lata I. Shukla[1], Amitava Adhikary[1,2], Robert Pazdro[1], David Becker[1] and Michael D. Sevilla[1,*]

[1]Department of Chemistry, Oakland University, Rochester, MI 38309, USA and [2]Department of Chemistry, Rajdhani College, University of Delhi, Raja Garden, Delhi 110 015, India

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ABSTRACT

Electron spin resonance (ESR) studies of radicals formed by radiation-induced multiple one-electron oxidations of guanine moieties in DNA are reported in this work. Annealing of gamma-irradiated DNA from 77 to 235 K results in the hydration of one electron oxidized guanine ($G^{\bullet+}$) to form the 8-hydroxy-7, 8-dihydroguanin-7-yl-radical (•GOH) having one β-proton coupling of 17–28 G and an anisotropic nitrogen coupling, $A_{\|}$, of ∼20 G, $A_{\perp} = 0$ with $g_{\|} = 2.0026$ and $g_{\perp} = 2.0037$. Further annealing to 258 K results in the formation of a sharp singlet at $g = 2.0048$ with line-width of 5.3 G that is identified as the 8-oxo-7,8-dihydroguanine one-electron-oxidized radical (8-oxo-$G^{\bullet+}$). This species is formed via two one-electron oxidations of •GOH. These two one-electron oxidation steps leading to the formation of 8-oxo-$G^{\bullet+}$ from •GOH in DNA, are in accordance with the expected ease of oxidation of •GOH and 8-oxo-G. The incorporation of oxygen from water in $G^{\bullet+}$ leading to •GOH and to 8-oxo-$G^{\bullet+}$ is verified by ESR studies employing ^{17}O isotopically enriched water, which provide unambiguous evidence for the formation of both radicals. ESR analysis of irradiated-DNA in the presence of the electron scavenger, Tl^{3+}, demonstrates that the cationic pathway leads to the formation of the 8-oxo-$G^{\bullet+}$. In irradiated DNA–Tl^{3+} samples, Tl^{3+} captures electrons. Tl^{2+} thus produced is a strong oxidant (2.2 V), which is metastable at 77 K and is observed to increase the formation of $G^{\bullet+}$ and subsequently of 8-oxo-$G^{\bullet+}$ upon annealing. We find that in the absence of the electron scavenger the yield of 8-oxo-$G^{\bullet+}$ is substantially reduced as a result of electron recombinations with $G^{\bullet+}$ and possible reaction with •GOH.

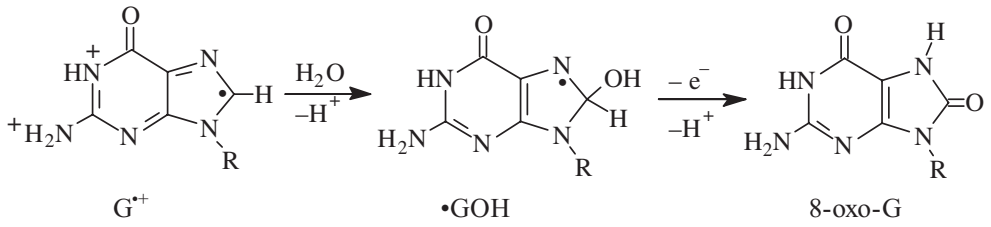
INTRODUCTION

One-electron oxidative damage to chromosomal and mitochondrial DNA is a subject of continuing interest owing to its implications in mutations, strand breaks and cell death (1–4). Among the DNA bases, guanine is the most easily oxidized (5–8) to its radical cation ($G^{\bullet+}$). The subsequent chemistry of $G^{\bullet+}$ in DNA is critical to understanding oxidative damage to DNA, and work in model systems has been helpful in the elucidation of these reactions. For example, the radical cation of 2′-deoxyguanosine ($dG^{\bullet+}$) in aqueous aerated solution is known to undergo transformation by two competitive reactions, i.e. hydration and deprotonation (5–8). The pK_a of $dG^{\bullet+}$ in 2′-deoxyguanosine is 3.9, hence at neutral pH, $dG^{\bullet+}$ undergoes fast deprotonation from N-1 (1.8×10^7 s^{-1}) in comparison to the slow hydration reaction at C-8 (5–8). In double-stranded DNA, base pairing induces a shift in the deprotonation equilibrium towards $G^{\bullet+}$ (2,8) and its lifetime is increased to be on the order of seconds whereas its lifetime is too short to be measured in a single-stranded DNA (9). Fast photochemical studies also show that the oxidized guanine moieties remain protonated immediately following electron transfer in duplex DNA (10). As a result of base pairing, nucleophilic addition of water at C-8 in $G^{\bullet+}$ is preferred in the double-stranded DNA over single-stranded DNA (11–15). This hydration reaction gives rise to a reducing radical, 8-hydroxy-7,8-dihydroguanin-7-yl-radical, (•GOH). Theoretical calculations point out that the energy needed for one-electron oxidation of •GOH is far lower than that of free 8-oxo-7,8-dihydroguanine (8-oxo-G) (13), which has an oxidation potential of −0.74 V at pH 7 (i.e. midpoint potential) (16). Thus, •GOH easily undergoes one-electron oxidation leading to 8-oxo-G as shown in Scheme 1 (14–22). We note that in a reducing environment, one-electron addition to •GOH may also occur; this leads to the ring opened product, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-G) (2,6,7,18).

In our previous work, 8-oxo-G was produced in DNA at room temperatures via oxidation with $Br_2^{\bullet-}$ (19). However, a plateau in the formation of 8-oxo-G (i.e. one 8-oxo-G per 127 ± 6 bp) was observed as a function of increasing $Br_2^{\bullet-}$ oxidation (19). The existence of this plateau was explained on the basis of substantially more favorable oxidation potential at pH 7 (i.e. midpoint potentials) of 8-oxo-G (−0.74 V) versus that of dG (−1.29 V) (5,6,11,13–20).

Since at pH 7,8-oxo-G is more easily oxidized than dG (5,6,11,13–20), sites containing 8-oxo-G in oligonucleotides are readily and selectively oxidized in preference to guanine moieties to produce a variety of oxidized products and their

*To whom correspondence should be addressed. Tel: +1 248 370 2328; Fax: +1 248 370 2321; Email: sevilla@oakland.edu



Scheme 1. Formation of 8-oxo-7,8-dihydroguanine from $G^{\bullet+}$ (R=H).

formation depends on the conditions under which the studies were carried out (23–30). For example, Duarte *et al*. (24) reported oxaluric acid as the major product of singlet oxygen-mediated oxidation of 8-oxo-G in the single-stranded DNA. On the other hand, cyanuric acid and oxaluric acid were formed as the major products of peroxynitrite-mediated damage of 8-oxo-G in the oligonucleotides (25,26). Oxidation of 8-oxo-G, using the iridium hexachloride complex, led to the formation of hydantoin derivatives (27,28). Several other products formed as a result of oxidation of 8-oxo-G are also reported in the literature (29–31).

As 8-oxo-G can be easily oxidized at pH 7, sites containing 8-oxo-G are expected to be a locus of hole transfer in DNA (13–21,32–36). Indeed, Shafirovich *et al*. (34) reported the direct observation of the formation of 8-oxo-G-radical cations in the double-stranded DNA via oxidation by 2-aminopurine-radical. Consecutive four-electron oxidation of guanine in the single- and double-stranded DNA by carbonate-radical anion has been reported recently (35); here, the radical intermediates of guanine and 8-oxo-G moieties in oligos were monitored by laser kinetic spectroscopy and the end-products of oxidation of guanine and 8-oxo-G moieties were identified (35). One ESR investigation of hole transfer and electron transfer to the oxidized products of guanine moieties in DNA at 77K shows that 8-oxo-G is a trap for the radiation-produced holes in DNA (19). Gas chromatography mass spectrophotometry (GC-MS) (17,22) and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)/MS reports (20) using ^{18}O-labeled water provide strong evidence for the hydration process shown in Scheme 1. However, there is no ESR report yet giving direct evidence for the radical intermediates shown in Scheme 1. For these reasons, we have chosen to investigate the mechanism of 8-oxo-G-radical formation from the guanine radical cation ($G^{\bullet+}$) in DNA in detail using ESR. The results reported in this work provide the evidence for the first time for the formation of each of the intermediates shown in Scheme 1 in gamma-irradiated hydrated DNA with and without electron scavengers in the absence of oxygen.

MATERIALS AND METHODS

Chemicals

Salmon testes DNA (sodium salt, 57.3% AT and 42.7% GC) was obtained from Sigma Chemical Company (St Louis, MO) and was used without further purification. Thallium trichloride and deuterium oxide (D_2O) (99.9%) were obtained from Aldrich Chemical Company Inc. (Milwaukee, WI). Water enriched with ^{17}O (59.9%) was purchased from Icon Services Inc. (NJ).

Preparations of DNA and DNA–Tl^{3+} samples

Homogenous DNA solutions were prepared in D_2O at concentrations of 100 mg/ml in the absence of oxygen. The DNA samples were lyophilized and then hydrated in D_2O as described in our previous studies (19,37–41).

Samples of DNA–Thallium trichloride (DNA–Tl^{3+}) at loading of one Tl^{3+} per 10, 20 and 35 bp were prepared by using equal volumes of DNA and thallium trichloride solutions—both in D_2O after they were thoroughly flushed with nitrogen. The DNA–Tl^{3+} samples were hydrated in D_2O or in Millipore H_2O (milli-Q) or in ^{17}O-enriched water [hydration (Γ) = 12 ± 2 in H_2O/nucleotide] as described in our previous work (19,37–41).

The hydrated DNA as well as DNA–Tl^{3+} samples were then pressed into cylinders i.e. pellets (0.4 cm × 1 cm height) in a nitrogen atmosphere using a teflon-coated aluminium dye and press and were immediately placed in liquid nitrogen.

Background Levels of 8-oxo-G

HPLC-EC analyses of the DNA employed in this work gave background levels of 15 ± 4 8-oxo-Gs per million nucleotides. Levels of at least 1/500 are needed to obtain detectable ESR signals (19).

Irradiation

The DNA and DNA–Tl^{3+} samples were exposed to ^{60}Co gamma rays with an absorbed dose of 1.6 kGy/h at 77 K. The gamma-irradiated samples were used for further studies.

Annealing of the samples

To carry out the temperature-dependent annealing of the samples, a variable temperature assembly was used under a dry nitrogen gas atmosphere. We have used a copper–constantan thermocouple to monitor the change in temperature. The samples were annealed for ~30 min or more at the given temperature.

After each annealing step, the samples were removed, immersed in liquid nitrogen and recorded at 77 K, which reduces temperature-dependent changes in spectra and maximizes signal height. This eliminates reversible changes in *A* and *g*.

Electron spin resonance

Electron spin resonance (ESR) spectra were recorded within a few minutes after irradiation and at increasing intervals thereafter at 77 K and at 45 or 40 dB (6.3 or 20 μW), digitized and stored in a 1000-point array on a microcomputer with field calibration marks from the three Fremy's salt (potassium

nitrosodiosulphonate) ESR lines (g = 2.0056, A_N = 13.09 G) similar to our earlier works (19,37–41).

Analysis of ESR spectra

As described in our previous studies (19,37–41), least square fittings of benchmark spectra were employed to determine the fractional composition of radicals in experimental spectra using programs (ESRPLAY, ESRADSUB) written in our laboratory. The fraction that a particular benchmark spectrum contributes to an overall spectrum is based on double integrated area, which is directly proportional to the spins of each species. The benchmark spectra used in this study include the guanine one-electron oxidized species in DNA ($G^{\bullet+}$), the DNA-base one-electron reduced species—$T^{\bullet-}$, $C^{\bullet-}$ which is likely to have been reversibly protonated at N3, i.e. $C(N3H)^{\bullet}$ and the product of irreversible protonation at C6 on $T^{\bullet-}$, i.e. TH• as well as the 8-oxo-G-radical are well established in the literature (19,37–42). The spectrum for the neutral radicals is that the remainder of all radicals found after the one-electron oxidized and reduced species above are scavenged (see Supplementary Figure S1); these neutral radicals are not further identified in this work but are attributed to sugar-backbone-radical species in part.

The important benchmark spectra for this work are those for 8-oxo-G-radical (19) and •GOH shown in Figure 1. •GOH in D_2O has one β-proton coupling of ∼17 G and an anisotropic nitrogen coupling, $A_{\|}$, of ∼20 G (Figure 1A) with $g_{\|}$ = 2.0026 and $g_{\perp}$ = 2.0037.

Analyses should be considered semiquantitative for intermediate species. However, the initial radical species at 77 K and the final species at 258 K are considered to be well-determined (±5%).

All irradiated hydrated-DNA samples and DNA–Tl^{3+} samples were run at power levels of 45 dB (6.3 μW) (39). Spectral simulations were carried out using SimFonia and WINEPR (Bruker).

RESULTS AND DISCUSSION

Annealing studies

We have carried out the temperature-dependent annealing of irradiated hydrated-DNA samples and of irradiated DNA–Tl^{3+} samples (Tl^{3+}, 1/10 bp) over the range 77–273 K. The resultant ESR spectra are shown in the Figure 2 for DNA and in Figure 3 for DNA–Tl^{3+} samples. The analyses of ESR spectra for radical components are shown in Figures 4 and 5. These analyses employed benchmark spectra shown in Figure 1 and from the literature (19,22,37–42,43).

The spectral populations of the radicals formed in the irradiated hydrated-DNA samples are found to be ∼40% $G^{\bullet+}$, ∼37% $DNA^{\bullet-}$ ($C^{\bullet-}$ and $T^{\bullet-}$) and ∼25% neutral radicals at 8.8 kGy dose (Figure 2A). The spectral populations in the irradiated DNA–Tl^{3+} sample in D_2O at 77 K (Figure 3A) are found to be ∼57% $G^{\bullet+}$, ∼5% $DNA^{\bullet-}$ and ∼23% neutral radicals (including sugar radicals) at 8.8 kGy dose. These approximate analyses suggest the possible formation of some •GOH in the irradiated DNA–Tl^{3+} sample using the benchmark spectrum (Figure 1A). However, experiments carried out using H_2O enriched with ^{17}O (59.9%) (*vide infra*) suggests that this species should comprise <5% of total radicals in irradiated DNA–Tl^{3+} samples in the temperature range 77–175 K.

Upon annealing at 195 K for 25 min, we observe in Figure 4 that the above-mentioned populations of the various

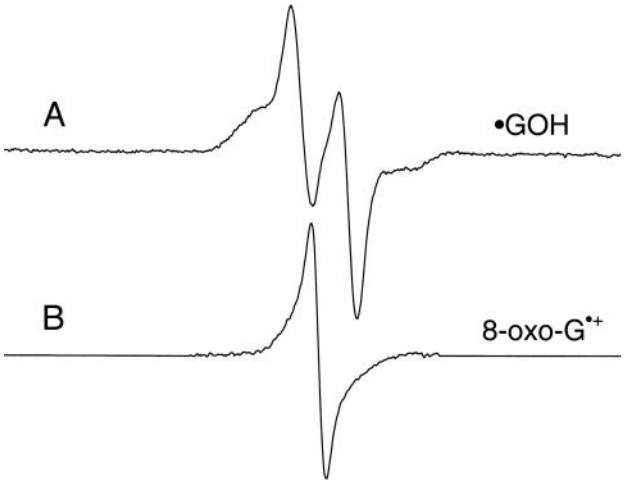


Figure 1. (**A**) ESR benchmark spectrum of •GOH in D_2O employed for analysis. The ESR spectra of gamma-irradiated DNA–Tl^{3+} samples in D_2O (Tl^{3+}, 1/10 bp) warmed to 195 K and subsequently to 235 K were recorded at 77 K. Computer subtraction of 70% of spectrum taken at 195 K was carried out from the spectrum obtained at 235 K and subsequent subtraction of 3% 8-oxo-$G^{\bullet+}$ spectrum [mentioned in (B)] from the remainder generates the benchmark spectrum of •GOH in D_2O. (**B**) The spectrum of 8-oxo-$G^{\bullet+}$ was obtained by photo-oxidation of 8-oxo-dG in frozen 8 M $NaClO_4$ aqueous (D_2O) solution (19). The three reference markers in this and in subsequent figures are Fremy's salt resonances (The central marker is at g = 2.0056 and each of three markers is separated from one another by 13.09 G).

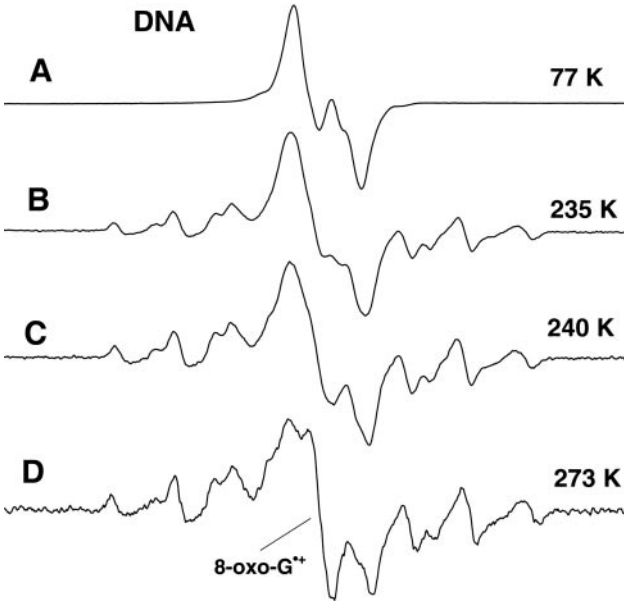


Figure 2. ESR spectra of gamma-irradiated (dose = 8.8 kGy) hydrated (D_2O)-DNA samples recorded at 77 K after annealing to different temperatures; (**A**) at 77 K, (**B**) at 235 K for 30 min, (**C**) at 240 K for 25 min, (**D**) at 273 K for 30 min.

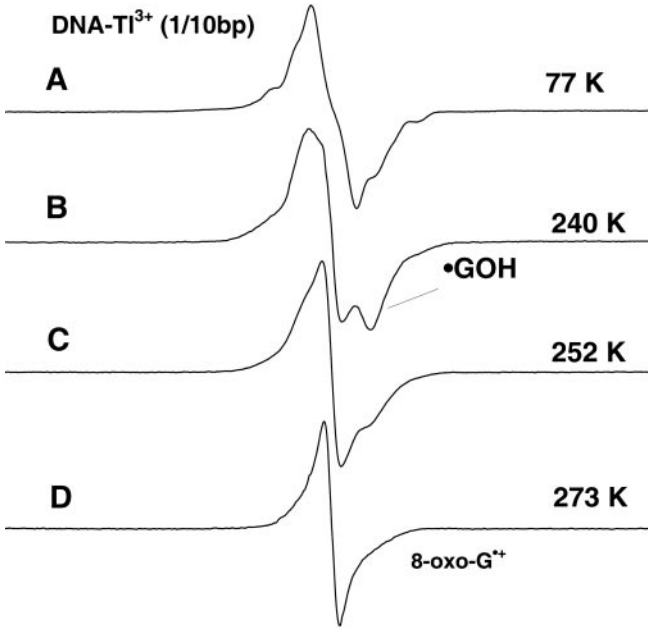


Figure 3. ESR spectra of gamma-irradiated (dose = 8.8 kGy) DNA–Tl^{3+} (Tl^{3+}, 1/10 bp) samples hydrated in D_2O only were recorded at 77 K after annealing to different temperatures; (**A**) at 77 K, (**B**) at 240 K for 30 min, (**C**) at 252 K for 30 min and (**D**) at 273 K for 30 min.

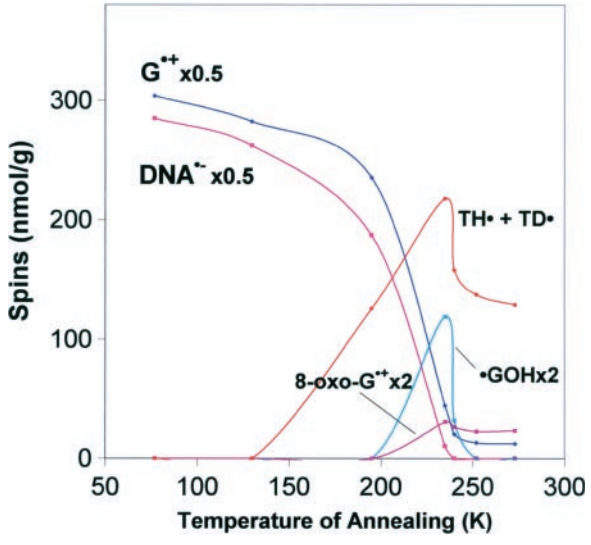


Figure 4. Variation of spins upon annealing temperature (77–273 K) for irradiated (dose =14.9 kGy) hydrated (D_2O only)-DNA samples as mentioned in Figure 2. Here, the spins of $G^{\bullet+}$ and $DNA^{\bullet-}$ are multiplied by 0.5; the spins of •GOH and 8-oxo-$G^{\bullet+}$are multiplied by 2 in order to make them prominent.

radicals react by: (i) electron hole recombination—as observed by loss of overall signal intensity as well as the decline in $G^{\bullet+}$ and $DNA^{\bullet-}$; and (ii) thymine anion protonation (and deuteration) at C6 to form TH• (TD•) as characterized by the well-known 8-line spectrum of TH• (38,40,42).

In contrast to hydrated-DNA samples, the spins for $G^{\bullet+}$ increase initially in irradiated DNA–Tl^{3+} samples (Figure 5) upon annealing from 77 to 195 K. The increase in $G^{\bullet+}$ results from one-electron oxidation of guanine moieties by the

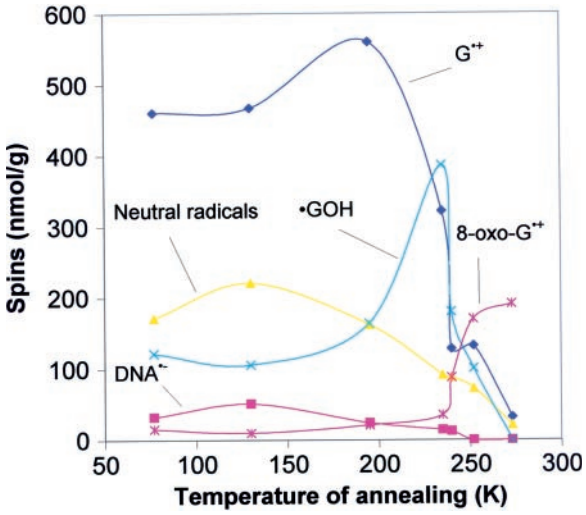


Figure 5. Variation of spins upon annealing temperature (77–273 K) of irradiated (irradiation dose = 8.8 kGy) DNA–Tl^{3+} samples (Tl^{3+}, 1/10 bp) hydrated in D_2O only. The ESR spectra of the corresponding samples were mentioned in Figure 3.

proximate Tl^{2+} formed as a result of the reduction of Tl^{3+} by excess electrons (44). ESR studies reveal that the anionic pathway is largely suppressed in irradiated DNA–Tl^{3+} samples. However, other than the obvious loss of the electron pathway, we did not detect this conversion of Tl^{3+} to Tl^{2+} by ESR at 77 K—because the Tl^{2+} is not apparent in the spectral region ($g = 2$) of our interest. The extent of loading of Tl^{3+} in DNA determines the fraction of radiation-produced electrons captured by Tl^{3+}. We have observed that for the loading of Tl^{3+} of 1/20 bp in DNA, the electron capture is almost complete. At irradiation doses approximately ⩾19 kGy, the amount of the anion spins observed at 77 K before annealing tends to increase and this is likely owing to the reduction of Tl^{3+} and its loss from DNA–Tl^{3+} system at higher doses (see Supplementary Figure S2).

Annealing irradiated DNA samples at 235 K, see Figures 2B and 4, results in a further loss in the spins of both $G^{\bullet+}$ and radical anions as well as the build up of the neutral radicals [TH• (TD•) and •GOH]. In the irradiated DNA–Tl^{3+} samples, upon annealing at 240 K, a loss in spins of $G^{\bullet+}$ corresponds to an increase in spins of •GOH. In addition, for DNA–Tl^{3+} samples we did not observe the formation of the 8-line spectrum characteristic of TH• (Figures 3B–D). This is in accordance with the complete suppression of the anionic pathway by Tl^{3+}. Since all our experiments on irradiated DNA and DNA–Tl^{3+} samples were performed under N_2-atmosphere, we did not observe peroxyl radical formation at ⩾190 K as observed earlier (42).

Annealing of the irradiated hydrated-DNA and DNA–Tl^{3+}-samples above 250 K for 30 min and more led to the loss of •GOH and the appearance of a sharp singlet ESR signal at $g = 2.0048$ with a line width of 5.3 G (Figures 2–5). Radical recombination results in a further decrease in the intensity of the ESR signal. In case of irradiated DNA–Tl^{3+} samples, the intensity of this sharp singlet accounts for ∼80% of the signal, whereas, in irradiated hydrated-DNA samples it accounts for ∼6% of spins. The identical ESR spectrum has been produced in another work by photo-oxidation of authentic 8-oxo-dG in

frozen 8 M $NaClO_4$ aqueous (D_2O) solution (19). We conclude that this sharp singlet is due to 8-oxo-G-radical formation. This singlet also closely matches the sharp singlet previously observed in X-band ESR spectrum during temperature-dependent annealing of X-irradiated 'dry DNA' (H_2O) and with low yield (42) as found in this work.

From the shapes of the curves in Figure 5 for $G^{\bullet+}$, •GOH and 8-oxo-$G^{\bullet+}$, we conclude that the conversion of $G^{\bullet+}$ into •GOH and finally 8-oxo-$G^{\bullet+}$ appears as a consecutive series of reactions (45).

The much lower yield of 8-oxo-G-radical in irradiated hydrated-DNA samples versus DNA–Tl^{3+} samples was attributed to the recombination of electrons with $G^{\bullet+}$ and possibly electron addition to •GOH resulting in Fapy-G (2,6-diamino-4-hydroxy-5-formamidopyrimidine). This reduces the yield of 8-oxo-G-radical via the oxidative pathway (2,6,7,18) shown in Scheme 1. In the case of irradiated DNA–Tl^{3+} samples, because of the electron capture by Tl^{3+}, the reductive pathway of damage is completely suppressed and yields of both •GOH and 8-oxo-G-radical are increased (*vide infra*).

Studies using $H_2{}^{17}O$

We have employed water enriched with ^{17}O (59.9%) to test the assumption that incorporation of an oxygen atom at C-8 of the $G^{\bullet+}$ to form •GOH and subsequently 8-oxo-G-radical is from DNA–water molecules of hydration. A matched set of two samples of hydrated DNA–Tl^{3+} (Tl^{3+}, 1/10 bp) one with ^{17}O enriched water of hydration and the other with ^{16}O water of hydration were irradiated at 77 K to a dose of 14.9 kGy. The spectra for the initial radicals formed at 77 K, chiefly $G^{\bullet+}$, and the final radical found at 258 K, 8-oxo-G radical for both samples are shown in Figure 6A and B, respectively. At 77 K, the ESR spectra of the samples with $H_2{}^{16}O$ and $H_2{}^{17}O$ are identical (Figure 6A) showing no significant matrix broadening

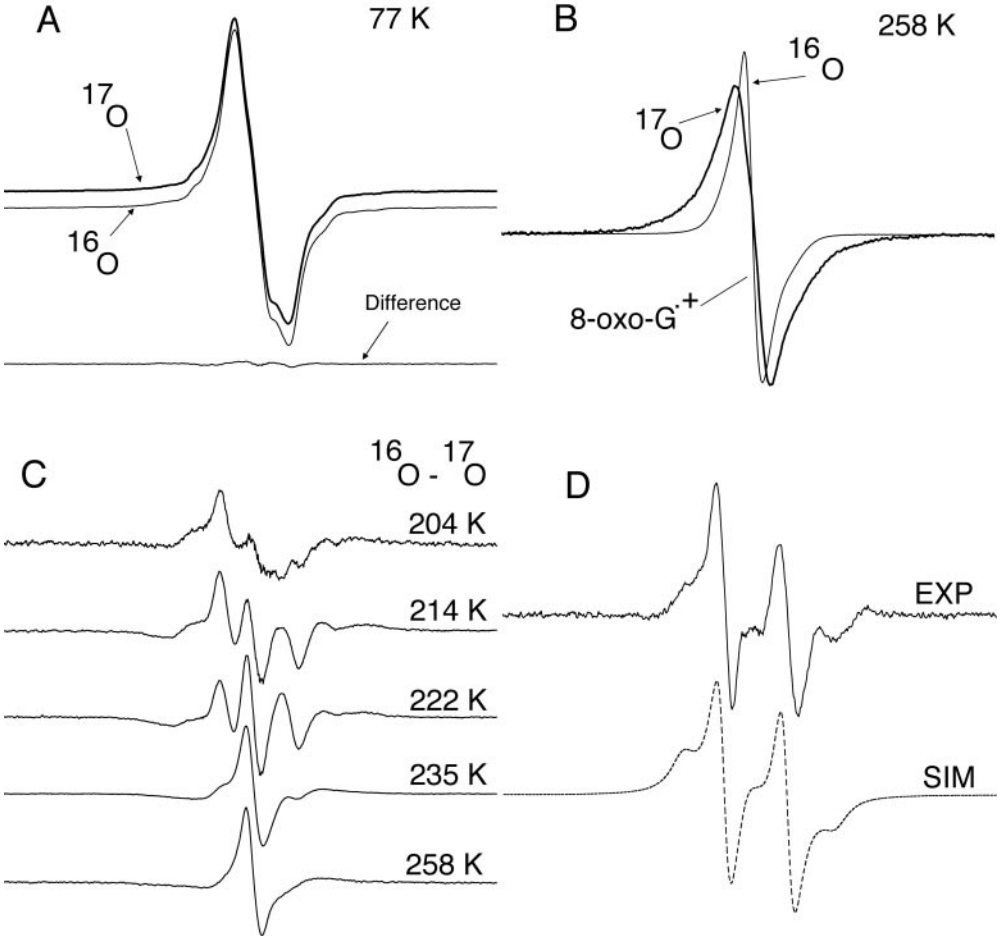
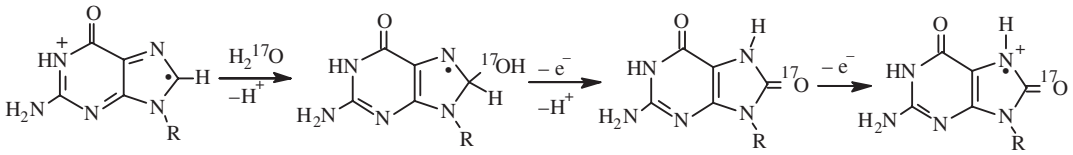
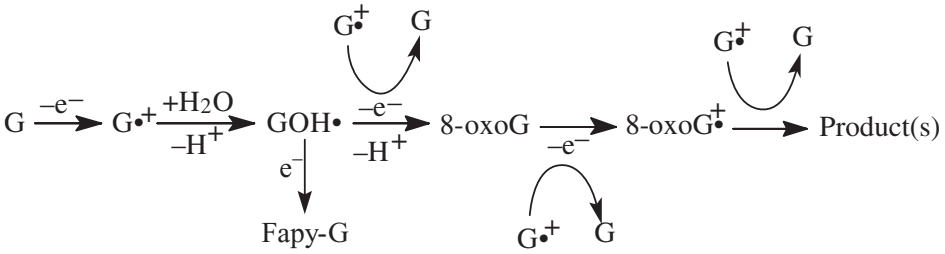


Figure 6. ESR spectra observed in irradiated (14.9 kGy) DNA–Tl^{3+} (Tl^{3+}, 1/10 bp) samples with and without water enriched with ^{17}O (59.9%). (**A**) At 77 K no difference in the spectra in $H_2{}^{16}O$ or $H_2{}^{17}O$ was observed as expected. The spectra in (A) are superimposable on each other; therefore, the spectrum of the $H_2{}^{17}O$ sample in (A) was lifted slightly to allow the spectrum of the $H_2{}^{16}O$ sample to be observed. (**B**) After 15 min of annealing at 258 K, we find a substantial difference in the two samples. Both spectra in (B) are assigned to the 8-oxo-G-radical (8-oxo-$G^{\bullet+}$). The spectrum in (B) for the sample enriched with ^{17}O has the appropriate amount of ^{16}O 8-oxo-G-radical spectrum subtracted (~55%). The increased breadth of the ^{17}O spectrum in B is attributed to unresolved ^{17}O hyperfine couplings and is clear evidence for the hydration reaction (Scheme 2). (**C**) Simple subtraction of the $H_2{}^{17}O$ spectra from the $H_2{}^{16}O$ spectra at each temperature from 204 K (where the hydration reaction to $G^{\bullet+}$ becomes apparent) to 258 K (where conversion to 8-oxo-G-radical is complete). (**D**) Upper spectrum is of •GOH from subtractions of the spectrum (76%) of the $H_2{}^{16}O$ sample annealed at 204 K and 12.5% of the same sample annealed at 258 K and a small, 2% of $G^{\bullet+}$ in $H_2{}^{16}O$ from that of the $H_2{}^{16}O$ sample annealed at 222 K. The lower spectrum is the computer simulated spectrum of •GOH obtained using the hyperfine parameters A(1H) = 28 G, A(1N) = (20, 0, 0) G, with g = 2.0026, 2.0037, 2.0037, and line width = (8, 6, 6) G.



Scheme 2. Mechanism of formation of 8-oxo-G-radical in gamma-irradiated DNA–Tl^{3+}-samples hydrated in H_2O enriched with ^{17}O (59.9%).



Scheme 3. Mechanism of formation of 8-oxo-$G^{\bullet+}$ and its further oxidation to end-products.

from the ^{17}O isotope as can be seen from the subtraction of the two spectra in the figure; however, at 258 K where 8-oxo-G-radical is formed, we find a much broader spectrum in the sample enriched with ^{17}O that we assign to unresolved ^{17}O couplings (Figure 6B). This is a strong evidence that no reaction occurs at 77 K and that at 258 K, the hydration reaction results in the incorporation of ^{17}O at C8 in 8-oxo-G-radical (Scheme 2). This is in complete agreement with the GC-MS (17,22) and MALDI-TOF/MS reports (20) using ^{18}O-labeled water and theoretical work (13,15). In Figure 6B, the spectrum for the sample enriched with ^{17}O has the appropriate subtraction of 8-oxo-G-radical with the ^{16}O isotope. The lack of resolution of the couplings is in agreement with simulations from theoretical calculations of the expected anisotropic hyperfine couplings. We also note that the spectrum of the 8-oxo-G-radical in ^{16}O water in Figure 6B is also somewhat broader than those in Figures 1B, 3C, 3D and 7C; this broadening is due to the additional unresolved hydrogen couplings since the spectra in Figures 1B, 3C, 3D and 7C were obtained in D_2O and not in H_2O.

A careful investigation of the intermediate temperatures for this matched set of one ^{16}O and ^{17}O sample was performed (Figure 6C). In a striking discovery, we found that simple subtraction of the $H_2{}^{17}O$ spectrum from the $H_2{}^{16}O$ spectrum at each temperature (Figure 6C) provided a spectrum dominated by the ^{16}O water addition radicals. This is because radicals produced by the addition of $H_2{}^{17}O$ are greatly broadened by the ^{17}O (nuclear spin = 5/2) hyperfine couplings and those from $H_2{}^{16}O$ addition become apparent in the subtractions. Further, all other radicals that do not undergo an addition reaction with water remain the same in both samples and hence they are cancelled in the subtraction. In Figure 6C, we show these subtractions from 204 K (where the hydration reaction to $G^{\bullet+}$ becomes apparent) to 258 K (where conversion to 8-oxo-G-radical is complete). The clear appearance of growth and decay of the doublet due to •GOH and the subsequent growth of the singlet due to 8-oxo-G-radical is evident in Figure 6C. This is the first clear demonstration of the formation of the intermediate •GOH in an unambiguous manner. We have also been able to isolate the spectrum of •GOH using suitable computer subtractions from the spectrum of the $H_2{}^{16}O$ sample annealed to 222 K (see Figure 6D). A simulation using the hyperfine parameters A(1H) = 28 G, A(1N) = (20, 0, 0) G, with g = 2.0026, 2.0037, 2.0037 and line width = (8, 6, 6) G, resulted in a spectrum very similar to that obtained by subtraction as mentioned above (Figure 6D). This spectrum matches well with a spectrum simulated using hyperfine-coupling constants reported previously for such a radical (42). On the other hand, •GOH in D_2O has one β-proton coupling of ~17 G and an anisotropic nitrogen coupling, an anisotropic nitrogen coupling, $A_{\|}$, of ~20 G, $A_{\perp}$ = 0 with $g_{\|}$ = 2.0026 and $g_{\perp}$ = 2.0037 (Figure 1A). We believe that different pK_a's on protonation/deuteration, may affect the conformation of the β-proton in •GOH and likely explains the difference in the β-proton hyperfine coupling in D_2O versus H_2O.

As •GOH is a highly reducing radical (13,16), we propose for irradiated DNA samples that subsequent one-electron oxidations of •GOH formed due to the hydration of $G^{\bullet+}$ lead to the formation of molecular 8-oxo-G and to 8-oxo-G radical (8-oxo-$G^{\bullet+}$ *vide infra*) by hole transfer in the absence of oxygen via Scheme 3.

The reduction potentials of the guanine moieties in the DNA polymer are not well established but one study points out that the reduction potential of $G^{\bullet+}$ moieties in plasmid DNA is 1.39 V at pH = 7 (midpoint potential) (46), which is slightly higher than that of $dG^{\bullet+}$ (1.29 V) in aqueous solution at pH 7 (2,5–8,13,16). Since stacking of 8-oxo-G with guanine moieties likely does not change the relative potential of guanine- and 8-oxo-G- moieties in DNA (14), the transfer of several holes to 8-oxo-G could be an effective self-protection mechanism for DNA molecule against radiation damage as multiple hole transfer to a single guanine site protects other bases from being oxidized. The observation that in intramolecular photoinduced electron transfer to anthraquinones linked to duplex DNA, an intervening 8-oxo-dG can act as a very efficient hole trap (33) supports this proposition. Moreover, evidence for sequence-specific damage leading to alkali labile sites in oligodeoxynucleotides containing 8-oxo-dG when gamma-irradiated under conditions of direct damage (47) also supports this proposition. In the solid state,

DNA holes have been shown to travel both along a primary duplex and between neighboring duplexes via tunneling, which encompasses tunneling from ca. 30 bp on the primary and neighboring duplexes at 77 K (43). Annealing activates hopping which extends the ranges substantially at ambient temperature holes and can range up to ~100 bp along a single DNA double strand (19,43,48–51). In the solid DNA samples used in this work transfer from adjacent strands would translate 100 bp along a single double-stranded DNA to encompass transfer from ~500 bp in closest proximity to the redox site at ambient temperatures. Such long-range transfer through gamma-irradiated DNA and DNA–Tl^{3+} upon annealing likely allows for the multiple one-electron oxidations of $G^{\bullet+}$ in DNA to 8-oxo-G-radical and beyond proposed in this work.

Comparison of the ESR characteristics of 8-oxo-G-radical obtained in our studies with existing data in the literature

Assuming that 8-oxo-G radical in DNA has the similar reduction potential value (0.74 V) as in free state in aqueous solution (14), the difference in the reduction potential at pH = 7 between $G^{\bullet+}$ (1.39 V) and DNA-8-oxo-G-radical (0.74 V) is 0.65 V. This difference corresponds to a large driving force for oxidation of 8-oxo-G by $G^{\bullet+}$ of 14.3 kcal/mol. It is clear in this study and also in other studies (2,11–36,43,46,47) that 8-oxo-G and its oxidation products are sinks for holes and/or oxidizing equivalents in DNA. Therefore, it is important that the 8-oxo-G-radical (the oxidized precursor from which various products are formed due to oxidation of 8-oxo-G) should be properly characterized. In Figure 7, we show the known experimental spectra (19) of one-electron reduced and oxidized radicals of 8-oxo-G, i.e. 8-oxo-$G^{\bullet-}$ (panel A) and 8-oxo-$G^{\bullet+}$ (panel B) along with the spectra of 8-oxo-G-radical we found in irradiated DNA samples (panel C). The structures of 8-oxo-$G^{\bullet-}$, 8-oxo-$G^{\bullet+}$ and its deprotonated species, 8-oxo-G($-H^{\bullet}$), are shown in Scheme 4.

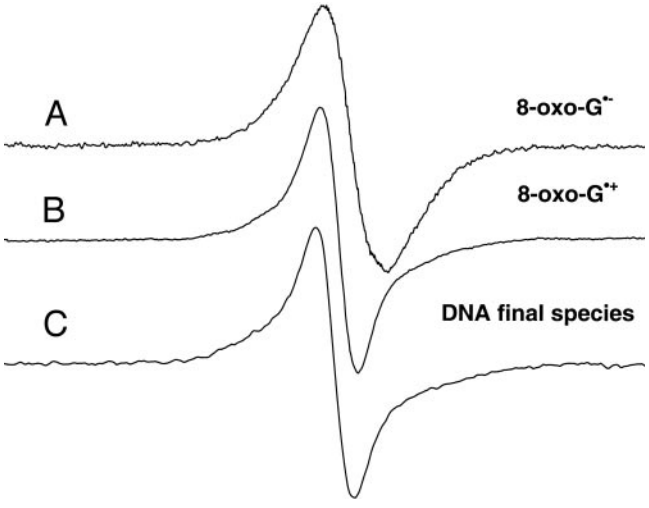


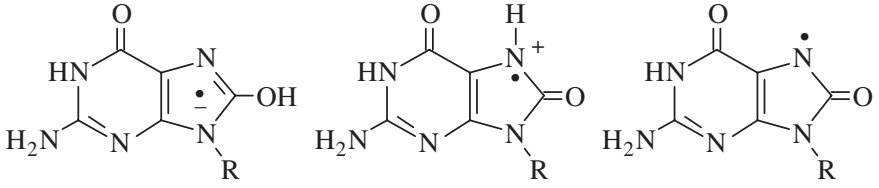
Figure 7. (**A**) The 8-oxo-G-radical anion (8-oxo-$G^{\bullet-}$) produced via one-electron addition due to γ-radiolysis of 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxo-dG) in frozen 7 M LiBr aqueous (D_2O) solutions (19). (**B**) The one-electron oxidized 8-oxo-G-radical (8-oxo-$G^{\bullet+}$) produced by photolysis of 8-oxo-dG in frozen 8 M $NaClO_4$ (D_2O) solution (4). (**C**) The 8-oxo-G-radical observed in irradiated (10.4 kGy) DNA–Tl^{3+} samples in D_2O (Tl^{3+}, 1/35 bp) warmed at 298 K for 25 min after subtraction of 10% of the $G^{\bullet+}$ spectrum.

Since, the pK_a of 8-oxo-$G^{\bullet+}$ = 6.6, we expect that the spectrum in panel B which is in a slightly acidic LiCl matrix is dominated by 8-oxo-$G^{\bullet+}$ (19) and not by the deprotonated, 8-oxo-G(-$H^{\bullet}$) (16). Optimized DFT calculations at B3LYP/6-31G(D) level show that the N-7 protonated species, 8-oxo-$G^{\bullet+}$, predicts hyperfine couplings more in keeping with the singlet ESR spectrum observed and thus also suggests that 8-oxo-$G^{\bullet+}$ accounts for our results in DNA. Theoretical calculations show that the N-7 protonated 8-oxo-$G^{\bullet+}$ species is favored over the 8-oxygen protonated enolic cationic structure by ~20 kcal/mol (15). From the similarities observed in the spectra in panels B and C, we conclude that we do not observe the one-electron reduced species, 8-oxo-$G^{\bullet-}$ as proposed by Cullis *et al*. (22), and instead we do observe the formation of one-electron oxidized 8-oxo-G in the form of the N-7 protonated 8-oxo-$G^{\bullet+}$ in irradiated DNA samples.

Dose–response of 8-oxo-G-radical

We also report here the dose dependence of 8-oxo-$G^{\bullet+}$ produced in the absence of oxygen in the irradiated hydrated-DNA as well as in the irradiated DNA–Tl^{3+} samples (Tl^{3+}, 1/20 bp) (Figure 8).

As expected, due to augmentation of cationic pathway of damage by Tl^{3+}, we observe higher spins for 8-oxo-$G^{\bullet+}$ in irradiated DNA–Tl^{3+} samples than in irradiated hydrated-DNA samples where the reductive pathways of damage leading to the recombination of ion radicals and to Fapy-G from •GOH compete with the oxidative pathway (2,6,7,18) (Scheme 3). We note that for a multiple oxidation process we might expect the dose response to curve upward with dose. Yet in Figure 8, we find saturation in the concentration of 8-oxo-$G^{\bullet+}$ at higher doses. This, we attribute to the expected further oxidation of 8-oxo-$G^{\bullet+}$ by higher concentrations of $G^{\bullet+}$ moieties in DNA formed at high doses.



Scheme 4. The structures of 8-oxo-$G^{\bullet-}$, 8-oxo-$G^{\bullet+}$ and 8-oxo-G(-$H^{\bullet}$).

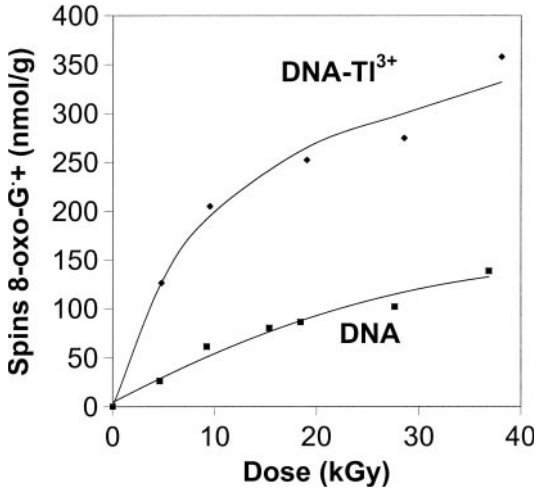


Figure 8. Dose response of spins of 8-oxo-$G^{\bullet+}$ formed upon annealing in irradiated hydrated [D_2O: H_2O (4.5:1, v/v)]-DNA samples and irradiated DNA–Tl^{+3} (1 Tl^{+3} /20 bp) samples hydrated in D_2O only at 258 K for ~1 h.

Since 8-oxo-$G^{\bullet+}$ is easily oxidized by $G^{\bullet+}$, 8-oxo-$G^{\bullet+}$ moieties in DNA are expected to collect additional holes from unreacted $G^{\bullet+}$ in DNA (Scheme 3). Further oxidation of 8-oxo-$G^{\bullet+}$ leads to the formation of end products as observed in other studies [see introduction and (11–36)] as indicated in Scheme 3, e.g. consecutive four-electron oxidation of guanine by carbonate radical anions led to spiroiminodihydantoin formation (35). Therefore, it is clear that one guanine site on the DNA strand may accept numerous holes once the process is facilitated by the hydration of $G^{\bullet+}$.

CONCLUSIONS

From the studies, our major findings are the following:

(i) *Detection of the 8-oxo-G-radical in the irradiated DNA*. We have shown earlier that at 77 K, 8-oxo-G is an effective hole-trap at low temperatures (19). This work shows that in the absence of oxygen, gamma-irradiated DNA results in the formation of one electron oxidized 8-oxo-G radical, 8-oxo-$G^{\bullet+}$. We find that the ESR line shape and *g*-value of this species in DNA are the same as those observed from an 8-oxo-$G^{\bullet+}$ formed by one-electron oxidation of authentic 8-oxo-dG (19). Moreover, our studies suggest that 8-oxo-G radical is the N-7 protonated radical cation rather than a radical-anion as reported earlier (22). We attribute an earlier report of a sharp singlet in irradiated DNA at 300 K (42) to 8-oxo-$G^{\bullet+}$.

(ii) *Mechanism of formation of 8-oxo-G-radical in the gamma-irradiated DNA*. Annealing of gamma-irradiated DNA–Tl^{3+} samples in the presence of water enriched with ^{17}O confirms that the conversion of $G^{\bullet+}$ into •GOH occurs by a hydration reaction. The conversion of $G^{\bullet+}$ into •GOH and finally to 8-oxo-$G^{\bullet+}$ by multiple one-electron oxidation in the absence of oxygen appears as a consecutive series of reactions (45).

Theoretical calculations point out that the energy needed for one-electron oxidation of •GOH is far lower than that of free 8-oxo-G (13) which has an oxidation potential value −0.74 V at pH 7 (i.e. midpoint potential) (16). Our annealing studies reveal that •GOH easily undergoes one-electron oxidations leading to 8-oxo-G and subsequently to 8-oxo-$G^{\bullet+}$; 8-oxo-$G^{\bullet+}$ also is easily oxidized so that collection of additional holes by 8-oxo-$G^{\bullet+}$ will lead to oxidized end-products as observed in other studies (11–36).

(iii) *Implication of these studies to self-protection of the DNA molecule against radiation damage*. The cationic pathway is considered to be the predominant source of DNA damage, and in gamma-irradiated solid-state-DNA holes can travel long distances both along a primary duplex and between neighboring duplexes via tunneling (43,48–51). Annealing activates hopping which extends the ranges substantially (43,48–51). As a result, the transfer of multiple holes to a single guanine site in gamma-irradiated DNA as shown in this study would be an effective self-protection mechanism for the DNA molecule against radiation damage as proposed earlier (33,35,47). This mechanism would be effective at high-radiation doses where oxidation sites from separate radiation tracks would be in proximity (<100 bp in distance). However, these doses are far beyond those of biological relevance (>300 Gy). We note that one radiation track may result in a single site with multiple damages, i.e. multiple damage sites (MDS) (52). MDS are known to be the most lethal type of radiation damage, which often places several oxidative damage sites within tunneling distances (53). The combination of several holes into a single lesion as proposed in this work would be an effective protection against this type of damage to the DNA molecule even at low doses.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR online.

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REFERENCES

1. Kim,J.E., Choi,S., Yoo,J.A. and Chung,M.H. (2004) 8-Oxoguanine induces intramolecular DNA damage but free 8-oxoguanine protects intermolecular DNA from oxidative stress. *FEBS Lett*., **556**, 104–110.
2. Burrows,C.J. and Muller,J.G. (1998) Oxidative nucleobase modifications leading to strand scissions. *Chem. Rev*., **98**, 1109–1152.
3. Bruner,S.D., Norman,D.P. and Verdine,G.L. (2000) Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature*, **403**, 859–866.
4. Lindahl,T. and Wood,R.D. (1999) Quality control by DNA repair. *Science*, **286**, 1897–1905.
5. Steenken,S. and Jovanovic,S.V. (1997) How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. *J. Am. Chem. Soc*., **119**, 617–618.
6. Steenken,S. (1989) Purine bases, nucleosides and nucleotides: aqueous solution redox chemistry and transformation reactions of their radical cations and e^- and OH adducts. *Chem. Rev*., **89**, 503–520.
7. Candeias,L.P. and Steenken,S. (2000) Reaction of $HO^{\bullet}$ with guanine derivatives in aqueous solution: formation of two different redox-active OH-adduct radicals and their unimolecular transformation reactions. Properties of $G(-H)^{\bullet}$. *Chem. Eur. J*., **6**, 475–484.
8. Kobayashi,K. and Tagawa,S. (2003) Direct observation of guanyl radical cation deprotonation in duplex DNA using pulse radiolysis. *J. Am. Chem. Soc*., **125**, 10213–10218.
9. Hildenbrand,K. and Schulte-Fröhlinde,D. (1990) ESR spectra of radicals of single-stranded and double-stranded DNA in aqueous solution.

Implications for •OH-induced strand breakage. *Free. Radic. Res. Commun.*, **11**, 195–206.
10. Lewis,F.D., Liu,X., Liu,J., Miller,S.E., Hayes,R.T. and Wasielewski,M.R. (2000) Direct measurement of hole transport dynamics in DNA. *Nature*, **406**, 51–53.
11. Angelov,D., Spassky,A., Berger,M. and Cadet,J. (1997) High-intensity UV laser photolysis of DNA and purine 2′-deoxyribonucleosides: formation of 8-oxopurine damage and oligonucleotide strand cleavage as revealed by HPLC and gel electrophoresis studies. *J. Am. Chem. Soc.*, **119**, 11373–11380.
12. Douki,T., Angelov,D. and Cadet,J. (2001) UV laser photolysis of DNA: effect of duplex stability on charge transfer efficiency. *J. Am. Chem. Soc.*, **123**, 11360–11366.
13. Reynisson,J. and Steenken,S. (2001) DFT calculations on the electrophilic reaction with water of the guanine and adenine radical cations. A for the situation in DNA. *Phys. Chem. Chem. Phys.*, **4**, 527–532.
14. Prat,F., Houk,K.N. and Foote,C.S. (1998) Effect of guanine stacking on the oxidation of 8-oxoguanine in B-DNA. *J. Am. Chem. Soc.*, **120**, 845–846.
15. Gervasio,F.L., Laio,A., Iannuzzi,M. and Parrinello,M. (2004) Influence of DNA structure on the reactivity of the guanine radical cation. *Chem. Eur. J.*, **10**, 4846–4852.
16. Steenken,S., Jovanovic,S.V., Bietti,M. and Bernhard,K. (2000) The trap depth (in DNA) of 8-oxo-7,8-dihydro-2′-deoxyguanosine as derived from electron transfer equilibria in aqueous solution. *J. Am. Chem. Soc.*, **122**, 2373–2374.
17. Kasai,H., Yamaizumi,Z., Berger,M. and Cadet,J. (1992) Photosensitized formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-hydroxy-2′-deoxyguanosine) in DNA by riboflavin: a non singlet oxygen mediated reaction. *J. Am. Chem. Soc.*, **114**, 9692–9694.
18. Ravanat,J., Saint-Pierre,C. and Cadet,J. (2003) One electron oxidation of the guanine moiety of 2′-deoxyguanosine: influence of 8-oxo-7,8-dihydro-2′-deoxyguanosine. *J. Am. Chem. Soc.*, **125**, 2030–2031.
19. Cai,Z. and Sevilla,M.D. (2003) Electron and hole transfer from DNA base radicals to oxidized product of guanine in DNA. *Radiat. Res.*, **159**, 411–419.
20. Misiaszek,R., Crean,C., Joffe,A., Geacintov,N.E. and Shafirovich,V. (2004) Oxidative DNA damage associated with combination of guanine and superoxide radicals and repair mechanisms via radical trapping. *J. Biol. Chem.*, **31**, 32106–32115.
21. Hickerson,R.P., Prat,F., Muller,J.G. and Burrows,C.J. (1999) Sequence and stacking dependence of 8-oxoguanosine oxidation: comparison of one electron vs. singlet oxygen mechanisms. *J. Am. Chem. Soc.*, **121**, 9423–9428.
22. Cullis,P.M., Malone,M.E. and Merson-Davies,L.A. (1996) Guanine radical cations are precursor of 7,8-dihydro-8-oxo-2′-deoxyguanosine but are not precursor of immediate strand breaks in DNA. *J. Am. Chem. Soc.*, **118**, 2775–2781.
23. Goyal,R.N. and Dryhurst,G. (1982) Redox chemistry of guanine and 8-oxyguanine and a comparison of the peroxidase-catalyzed and electrochemical oxidation of 8-oxyguanine. *J. Electroanal. Chem.*, **135**, 75–91.
24. Duarte,V., Gasparutto,D., Yamagucho,L.F., Ranavat,J.L., Martinez,G.R., Mascio,P.D. and Cadet,J. (2000) Oxaluric acid as the major product of singlet oxygen mediated oxidation of 8-oxo-7,8-dihydroguanine in DNA. *J. Am. Chem. Soc.*, **122**, 12622–12628.
25. Burney,S., Niles,J.C., Dedon,P.C. and Tannenbaum,S.R. (1999) DNA damage in deoxynucleoside and oligonucleotides treated with peroxynitrite. *Chem. Res. Toxicol.*, **12**, 513–520.
26. Tretyakova,N.Y., Niles,J.C., Burney,S., Wishnok,J.S. and Tannenbaum,S.R. (1999) Peroxynitrite induced reaction of synthetic oligonucleotide containing 8-oxoguanine. *Chem. Res. Toxicol.*, **12**, 459–466.
27. Luo,W., Muller,J.M., Rachlin,E.M. and Burrows,C.J. (2001) Characterization of hydantoin products from one electron oxidation of 8-oxo-7,8 dihydroguanosine in a nucleoside model. *Chem. Res. Toxicol.*, **14**, 927–938.
28. Luo,W., Muller,J.M., Rachlin,E.M. and Burrows,C.J. (2000) Characterization of spiroiminodihydantoin as a product of one-electron oxidation of 8-oxo-7,8-dihydroguanosine. *Org. Lett.*, **2**, 613–616.
29. Niles,J.C., Burney,S., Singh,S.P., Wishnok,J.S. and Tannenbaum,S.R. (1999) Peroxynitrite reaction products of 3′5′di-O-acetyl-8-oxo-7, 8-dihydro-2′-deoxyguanosine. *Proc. Natl Acad. Sci. USA*, **96**, 11729–11734.
30. Sheu,C. and Foote,C.S. (1995) Photosensitized reaction of 8-oxo-7,8-dihydro-2′-deoxyguanosine: derivative. Formation of dioxetane and hydroperoxide intermediates. *J. Am. Chem. Soc.*, **117**, 474–477.
31. Luo,W., Muller,J.M. and Burrows,C.J. (2001) The pH-dependent role of superoxide in riboflavin-catalyzed photo-oxidation of 8-oxo-7,8-dihydroguanosine. *Org. Lett.*, **3**, 2801–2804.
32. Ly,D., Sanii,L. and Schuster,G.B. (1999) Mechanism of charge transport in DNA: internally linked anthraquinone conjugates support photon-assisted polaron hopping. *J. Am. Chem. Soc.*, **121**, 9400–9410.
33. Gasper,S.M. and Schuster,G.B. (1997) Intramolecular photoinduced electron transfer to anthroquinones linked to duplex DNA: effect of gaps and traps on long range of radical cation migration. *J. Am. Chem. Soc.*, **119**, 12762–12771.
34. Shafirovich,V., Cadet,J., Gasparutto,D., Dourandin,A. and Geacintov,N.E. (2001) Direct spectroscopic observation of 8-oxo-7,8-dihydro-2′-deoxyguanosine radicals in double-stranded DNA generated by one-electron oxidation at a distance by 2-aminopurine radicals. *J. Phys. Chem. B*, **105**, 586–592.
35. Jaffe,A., Geactintov,N.E. and Shafirovich,V. (2003) DNA lesions derived from the site selective oxidation of guanine by carbonate radical anions. *Chem. Res. Toxicol.*, **16**, 1528–1538.
36. Shafirovich,V., Cadet,J., Gasparutto,D., Dourandin,A. and Geacintov,N.E. (2001) Nitrogen dioxide as an oxidizing agent of 8-oxo-7,8-dihydro-2′-deoxyguanosine but not of 2′-deoxyguanosine. *Chem. Res. Toxicol.*, **14**, 233–241.
37. Messer,A., Carpenter,K., Forzley,K., Buchanan,J., Yang,S., Razskazovkii,Y., Cai,Z. and Sevilla,M.D. (2000) Electron spin resonance study of electron transfer rates in DNA: determination of the tunneling constant for single-step excess electron transfer. *J. Phys. Chem. B*, **104**, 1128–1136.
38. Wang,W. and Sevilla,M.D. (1994) Protonation of nucleobase anions in gamma-irradiated DNA and model systems. Which DNA base is the ultimate sink for the electron? *Radiat. Res.*, **138**, 9–17.
39. Sevilla,M.D., Becker,D., Yan,M. and Summerfield,S.R. (1991) Relative abundances of primary ion radicals in γ-irradiated DNA: cytosine vs thymine anions and guanine vs adenine cations. *J. Phys. Chem.*, **95**, 3409–3415.
40. Wang,W., Yan,M., Becker,D. and Sevilla,M.D. (1994) The influence of hydration on the absolute yields of primary free radicals in gamma-irradiated DNA at 77 K. II. Individual radical yields. *Radiat. Res.*, **137**, 2–10.
41. Wang,W. and Sevilla,M.D. (1994) Reaction of cystamine with individual DNA base radicals in gamma-irradiated nucleotides at low temperature. *Int. J. Radiat. Biol.*, **66**, 683–695.
42. Weiland,B. and Hüttermann,J. (1998) Free radicals from X-irradiated 'dry' and hydrated lyophilized DNA as studied by electron spin resonance spectroscopy: analysis of spectral components between 77 K and room temperature. *Int. J. Radiat. Biol.*, **74**, 341–358.
43. Cai,Z. and Sevilla,M.D. (2004) Studies of excess electron and hole transfers. In Schuster,G.B. (ed.), *In Long Range Charge Transfer in DNA II. Topics In Current Chemistry*. Springer-Verlag, Berlin, Germany, Vol. 237, pp. 103–127.
44. Schwarz,H.A. and Dodson,R.W. (1984) Equilibrium between hydroxyl radicals and thallium (II) and the oxidation potential of OH (aq). *J. Phys. Chem.*, **88**, 3643–3647.
45. Eggers,D.F.,Jr, Gregory,N.W., Halsey,G.D.,Jr and Rabinovitch,B.S. (1963) *Physical Chemistry*. John Wiley and Sons, Inc., New York, London.
46. Milligan,J.R., Aguilera,J.A. and Ward,J.F. (2001) Redox equilibrium between guanyl radicals and thiocyanate influences base damage yields in gamma irradiated plasmid DNA. Estimation of the reduction potential in guanyl radicals in plasmid DNA in aqueous solution at physiological ionic strength. *Int. J. Radiat. Biol.*, **77**, 1195–1205.
47. Doddridge,Z.A., Cullis,P.M., Jones,G.D.D. and Malone,M.E. (1998) 7,8-Dihydro-8-oxo-2′-deoxyguanosine residues in DNA are radiation damage ''hot'' spots in the direct γ radiation damage pathway. *J. Am. Chem. Soc.*, **120**, 10998–10999.
48. Delaney,S. and Barton,J.K. (2003) Long-range DNA charge transport. *J. Org. Chem.*, **68**, 6475–6483.

49. Davis,W.B., Naydenova,I.I., Haselsberger,R., Ogrodnik,A., Giese,B. and Michel-Beyerle,M.E. (2000) Dynamics of hole trapping by G, GG, and GGG in DNA. *Angew. Chem. Int. Ed.*, **39**, 3649–3652.
50. Bixon,M., Giese,B., Wessely,S., Langenbacher,T., Michel-Beyerle,M.E. and Jortner,J. (1999) Long-range charge hopping in DNA. *Proc. Natl Acad. Sci. USA*, **96**, 11713–11716.
51. Giese,B. (2002) Long-distance electron transfer through DNA. *Annu. Rev. Biochem.*, **71**, 51–70.
52. Ward,J.F. (1994) The complexity of DNA damage: relevance to biological consequences. *Int. J. Radiat. Biol.*, **66**, 427–432.
53. Milligan,J.R., Aguilera,J.A., Paglinawan,R.A., Nguyen,K.J. and Ward,J.F. (2002) Modification of ionizing radiation clustered damage: estimate of the migration distance of holes through DNA via guanyl radicals under physiological conditions. *Int. J. Radiat. Biol.*, **78**, 733–741.