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Spectral Characterization of Guanine C4-OH Adduct: A Radiation and Quantum Chemical Study

Suvarna D. Phadatare,[†] Kiran Kumar K. Sharma,[‡] B. S. M. Rao,[†,§] S. Naumov,[∥] and Geeta K. Sharma*[,†]

[†]National Centre for Free Radical Research (NCFRR), Department of Chemistry, University of Pune, 411007, Maharashtra, India

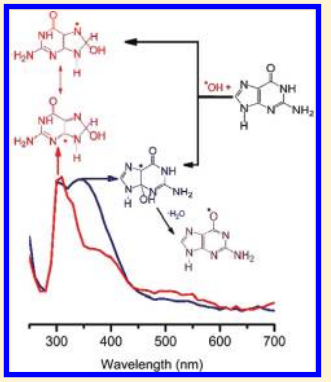
[‡]School of Chemical Sciences, Department of Physical Chemistry, North Maharashtra University, Jalgaon, 425001, Maharashtra, India

[§]Department of Chemistry, Indian Institute Science Education and Research (IISER), Pune 411021, India

[∥]Leibniz-Institut für Oberflächenmodifizierung e. V. (IOM), Permoserstr. 15, D-04318 Leipzig, Germany

Supporting Information

ABSTRACT: The reaction of hydroxyl radical ($^{\bullet}$OH) with guanine was investigated under restricted pH condition (pH 4.6) using pulse radiolysis technique. The time-resolved optical transient absorption spectra showed two peaks centered at 300 and 330 nm at 4 μs after the pulse which exhibited different reactivity toward molecular oxygen (O_2). The peak at 300 nm was found to be relatively more stable than the peak at 330 nm. The peak corresponding to 330 nm decayed within 20 μs having a first order rate constant 4−7 × 10^4 s^{-1} and was pH dependent. On longer time scale, the species decayed by a bimolecular process. The presence of O_2 did not affect its decay rate constant. The $^{\bullet}$OH reacts with guanine at pH 4.6 with a diffusion-controlled second order rate constant of ≥1 × 10^{10} mol^{-1} dm^3 s^{-1}. The reaction of $Br_2^{\bullet-}$, $O_2^{\bullet-}$, and 2-hydroxy-2-propyl radical with guanine was also investigated to differentiate among the one-electron oxidized, one-electron reduced species of guanine and the guanine−OH adducts formed in the reaction of $^{\bullet}$OH at pH 4.6. On the basis of the spectral characteristics and reactivity toward O_2, two guanine−OH adduct species were identified (i) the C4-OH adduct species absorbing at 330 nm which has not been reported so far and (ii) the C8-OH adduct species absorbing at 300 nm in agreement with the known literature absorption features. Quantum chemical calculations using BHandHLYP with 6-31+G(d,p) basis set and excited state calculations using TDDFT for all possible transients complement the assignment of the observed spectral peak at 330 nm to the C4-OH adduct of guanine. Furthermore, steady state radiolysis revealed the formation of 8-hydroxy-guanine whose precursor is known to be the C8-OH adduct species.



INTRODUCTION

An understanding of the mechanism of the $^{\bullet}$OH reaction with guanine moiety (structure **I**, Scheme 1) has been the focus of intense research interest (for recent reviews, see refs 1−4 and the references cited therein). It has drawn special attention of radiation chemists possibly because of its low redox potential among the deoxyribonucleic acid (DNA) nucleobases.[5,6] The decay of the transient species formed in the $^{\bullet}$OH reaction with guanine moiety generates nonradical products like 2,6-diamino-4-hydroxy-5-formamido-pyrimidine (FaPyGua) and 8-oxo-7,8-dihydroguanine (8-oxo-G), even in anoxic condition, affecting the normal functioning of the DNA.[7−9]

The initial pulse radiolysis studies by O'Neill and co-workers[10,11] on the reaction of $^{\bullet}$OH with 2′-deoxyguanosine and the 5′-monophosphate derivative showed the production of nearly equal amount of oxidizing and reducing type radicals and suggested $^{\bullet}$OH addition to the C4 as a reaction pathway. These findings were critically reviewed by Steenken[12] and further a detailed investigation[13] on 2′-deoxyguanosine and derivatives by his group confirmed the addition of $^{\bullet}$OH as the major process.

The mechanism involved ∼60−70% $^{\bullet}$OH addition to the C4 position (structure **II**, Scheme 1) and ∼17% was attributed to the formation of the C8-OH adduct of guanine moiety (structure **III**). The former subsequently undergoes tautomerization reaction which was assigned to water elimination reaction resulting in the formation of the oxidizing radical **V**. The findings dovetail the investigation[14] by the same group on the $^{\bullet}$OH reaction with adenine and its derivatives. The spectral features for the $^{\bullet}$OH reaction were found to be similar in nucleobase adenine and its nucleoside and nucleotide. It can be concluded from these results that the substituent at N9 does not seem to play a role. Recent theoretical calculations[15] on the $^{\bullet}$OH reaction with adenine, however, suggested H-abstraction as an important pathway.

Interestingly, Chatgilialoglu and co-workers[16−19] from their recent experimental and theoretical studies on guanosine and derivatives have also shown H-abstraction is predominant, instead of addition. Their conclusions were based on a detailed investigation of the reactions of $^{\bullet}$OH with guanosine, 2′-deoxyguanosine and derivatives[19] and hydrated electron with 8-bromoguanosine.[16] According to them, the H-abstraction from the amino group results

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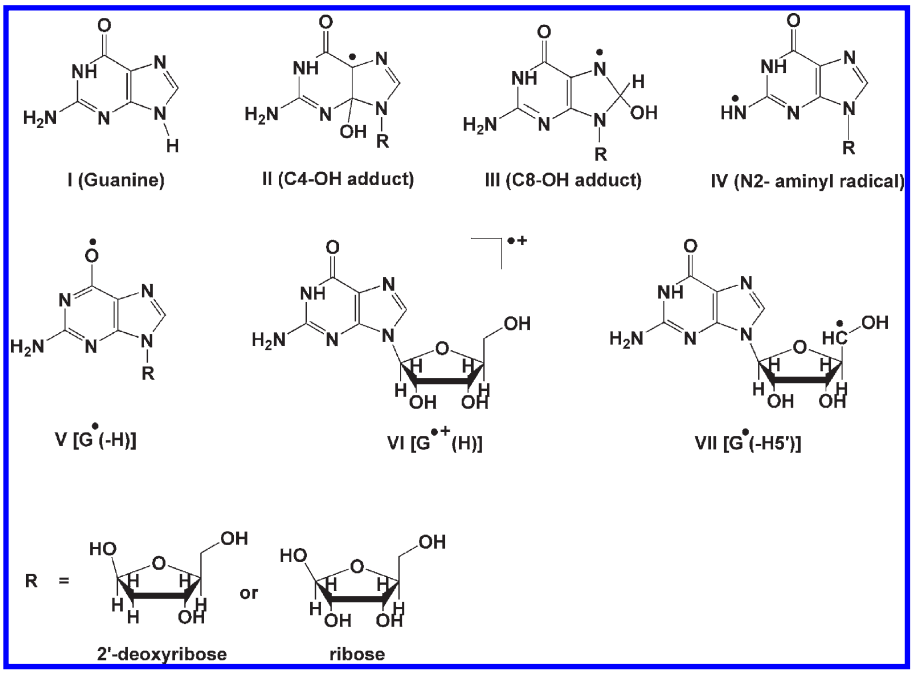
Scheme 1. Various Intermediate Products Reported[13,19,20] in the $^{\bullet}$OH Reaction with Guanosine and Derivatives



in the formation of aminyl radical **IV** (Scheme 1). Subsequently, it undergoes water assisted tautomerization to form the more stable guanyl radical **V**. These findings were complemented by their time-dependent DFT calculations for the guanosine and 2′-deoxyguanosine radicals where an excellent agreement with the predicted optical transition bands was seen.

On the other hand, a different mechanism involving the sequential electron proton transfer (SEPT) was proposed by Galano and Alvarez-Idaboy[20] from their theoretical study. The SEPT results in the formation of the radical cation (**VI**) in the first step followed by the radical **VII** on deprotonation at C5′- of the sugar moiety (Scheme 1).

It is thus evident that the nature of the precursor species for the formation of gunyl radical **V** (Scheme 1) is not yet clearly understood. Thus, there is a need to revisit the radiation chemical oxidation of guanine. Guanine was chosen as a target molecule because it has not been studied in detail so far owing to its intrinsic insolubility associated in aqueous medium with only the second order rate constant for its reaction with $^{\bullet}$OH with guanine having been determined[21] by competitive kinetics at pH 10. In addition, the pK_a values of guanine have been determined both experimentally and from theoretical calculations (see ref 22 and the references cited therein) and the three pK_a's corresponding to N9, N1, and N7 are 12.4, 9.4, and 3.3, respectively, with the last corresponding to the N7-tautomeric proton. Therefore, at pH of 3.8−4.6, the structure of guanine (Structure **I**, Scheme 1) will constitute >75−99%. Further, in the case of guanine the deprotonation from the sugar moiety, as seen in the guanosine and its derivatives is not operative, though H-abstraction from N9- is additional possibility.

This investigation is undertaken with a view to gain further insight into the mechanism of the $^{\bullet}$OH radical reaction and examines the nature of the precursor for the formation of the relatively more stable radical **V** (Scheme 1) which is well established.[13,19] Herein, we report the study of kinetics, the transient absorption spectra and the properties of the intermediates formed in the reactions of $^{\bullet}$OH, $Br_2^{\bullet-}$, $O_2^{\bullet-}$, and 2-hydroxy-2-propyl radicals with guanine using pulse radiolysis and product analysis by HPLC under steady state conditions. The study is also complemented by theoretical studies which support the experimental data.

MATERIALS AND METHODS

Chemicals. Nucleobase guanine was from sigma. All of the solutions were prepared in deionized water from Milli-Q system. Highly pure Iolar grade nitrous oxide and oxygen gas were from Inox. All other chemicals used were either from Qualigens or Merck. A Shimadzu-1650 UV−VISIBLE spectrophotometer was used for spectral analysis and Eutech instrument pH 510 m was used for monitoring the pH of the solution.

Preparation of Guanine Solution. Appropriate quantity of guanine nucleobase was taken and first dissolved with a minimum quantity of conc. HCl. This mixture was diluted with Ultrapure Milli-Q water. The pH was then adjusted using NaOH solution. The final concentration of dissolved guanine was determined from the OD at 246 nm using the extinction coefficient (10.7×10^3 dm^3 mol^{-1} cm^{-1}) of guanine nucleobase.[23]

Pulse Radiolysis. The pulse radiolysis experiment was carried out using a 7 MeV linear accelerator at the Pune University Linear Accelerator Facility (PULAF) which delivers a 50 ns pulse electron beam with a dose rate of 3−4 Gy/pulse at 23 °C, as determined by using aerated 1×10^{-2} mol dm^{-3} potassium thiocyanate dosimeter at 480 nm, taking $G\varepsilon_{480\ nm} = 2.6 \times 10^{-4}$ m^2 J^{-1} for $(SCN)_2^{\bullet-}$.[24,25] The dose rate was kept low to avoid bimolecular processes involving the transient species produced. The details of the facility are reported elsewhere.[26] The transient spectra were recorded by using a N_2O saturated aqueous solution of guanine in a flow system.

Steady State Studies. Steady state studies were carried out by using a well-type ^{60}Co γ-source at the Department of Chemistry, University of Pune.[27] The dose rate determined using Fricke's dosi meter was 4.7 Gy/min. The solution containing guanine nucleobase

in a test tube was saturated with N_2O for 30 min and was exposed to the source for the time span calculated for the amount of absorbed dose required. The test tube was secured with rubber septum during the N_2O purging and irradiation.

Irradiations. Radiolysis of aqueous solution with γ-radiation or highly energetic electrons leads to the generation of extremely reactive species (Reaction 1), the yields (G-values) of which are well documented.

$$H_2O \rightsquigarrow {}^{\bullet}OH,\ e_{aq}^{-},\ H^{\bullet},\ H_2O_2,\ H_2,\ H_2O^{+} \quad (1)$$

$G^{\bullet}_{OH} = G_{e_{aq}^-} = 0.28$; $G^{\bullet}_{H} = 0.055 \times 10^{-6}$ mol J^{-1}.

e_{aq}^{-} ($E(H_2O/e_{aq}^{-}) = -2.87$ V) are quantitatively converted into ${}^{\bullet}OH$ ($pK_a = 11.9$)[28] by saturating the aqueous solution with N_2O (reaction 2).

$$e_{aq}^{-} + N_2O \xrightarrow{H_2O} {}^{\bullet}OH + N_2 + OH^{-} \quad (2)$$

$k = 8.7 \times 10^9$ mol dm^{-3} s^{-1} at pH 7[28]

2-Hydroxy-2-propyl radicals ($E((CH_3)_2CHOH/(CH_3)_2C^{\bullet}-OH) = -1.8$ V, $pK_a \sim 12.2$)[4] were generated by quantitative conversion of OH and H radicals under N_2O conditions containing 0.1 mol dm^{-3} of 2-propanol (reactions 3 and 4)

$$ {}^{\bullet}OH + (CH_3)_2HCOH \xrightarrow{N_2O} {}^{\bullet}COH(CH_3)_2 + H_2O \quad (3)$$

$k = 1.9 \times 10^9$ mol dm^{-3} s^{-1}.[28]

$$(CH_3)_2HCOH + {}^{\bullet}H \xrightarrow{N_2O} {}^{\bullet}COH(CH_3)_2 + H_2 \quad (4)$$

Bromine radical anion ($E(Br_2^{\bullet-}/2\ Br^-) = 1.66$ V)[29] was produced by irradiating N_2O saturated solution containing 0.1 mol dm^{-3} KBr where OH radical are quantitatively converted into $Br_2^{\bullet-}$ (reactions 5 and 6).[30]

$$ {}^{\bullet}OH + Br^- \rightarrow Br^{\bullet} + OH^- \quad (5)$$

$k = 1.1 \times 10^{10}$ mol dm^{-3} s^{-1}.

$$Br^{\bullet} + Br^- \rightarrow Br_2^{\bullet-} \quad (6)$$

$k = 1.2 \times 10^{10}$ mol dm^{-3} s^{-1}.

Superoxide radical anion ($O_2^{\bullet-}$), with a redox potential of $\sim$1.0 V,[29] was generated by pulse radiolysis of O_2 saturated solution containing 10×10^{-6} mol dm^{-3} of ethylene diamine tetraethyl acetate (EDTA) and 0.1 mol dm^{-3} sodium formate (reactions 7–9).[30] EDTA is added as chelating agent to remove any transient metals to prevent quenching of the $O_2^{\bullet-}$.

$$e_{aq}^{-} + O_2 \rightarrow O_2^{\bullet-} \quad (7)$$

$k = 1.9 \times 10^{10}$ mol dm^{-3} s^{-1}.

$$ {}^{\bullet}OH + HCOO^- \rightarrow CO_2^{\bullet-} \quad (8)$$

$k = 1.87 \times 10^{10}$ mol dm^{-3} s^{-1}.

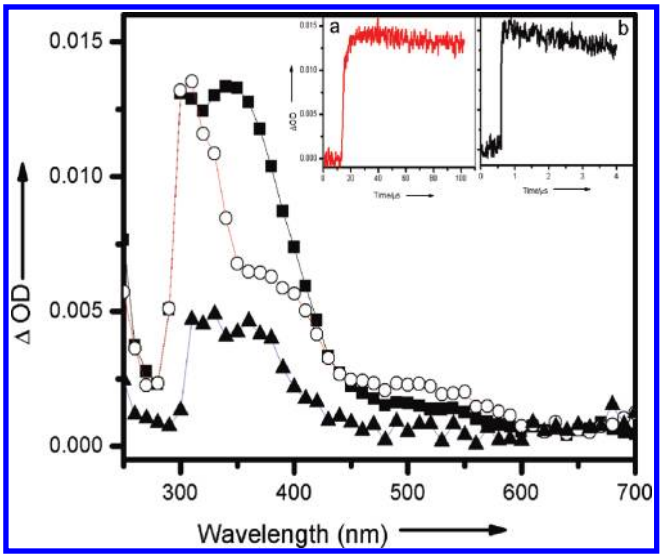


Figure 1. Time-resolved transient optical absorption spectra obtained from the pulse radiolysis of N_2O-saturated solution containing 0.5×10^{-3} mol dm^{-3} guanine at pH 4.6 recorded at 4 (■), 20 (○), and 800 μs (▲) after the pulse dose/pulse = 3–4 Gy. Inset: Absorption at (a) 300 and (b) 330 nm.

$$CO_2^{\bullet-} + O_2 \rightarrow O_2^{\bullet-} + CO_2 \quad (9)$$

$k = 3.2 \times 10^9$ mol dm^{-3} s^{-1}.

Acid Hydrolysis of 8-Hydroxy-2-deoxyguanosine. A total of 100 μL of 1 μg/mL 8-hydroxy-2-deoxyguanosine was saturated with high purity Ar gas and was baked at 90 °C for 90 min. The procedure followed was similar with the acid hydrolysis of irradiated DNA used for the analysis of base damage products by GC-MS,[31] except that hydrolysis was not carried out in a vacuum sealed tube. The residue obtained was dissolved in 100 μL of ammonium acetate buffer (pH 4.00) and 20 μL of the solution was injected for the HPLC analysis.

HPLC Analysis. A Shimadzu Gradient HPLC system (Shimadzu Prominence 20A) interfaced to a UV-photomultiplier detector was used for product analysis. The method employed was similar to that reported for the separation of DNA free bases,[32] except for the few modification as follows. The column used was a PrincetonSPHER-100 C-18 reverse-phase column (4.6 × 150 mm, 10 μm, 100 Å pore size) at 30 °C, and the mobile phase (40 mM ammonium acetate) was adjusted to pH 4.00 using acetic acid so as to separate 8-oxo-guanine from other nucleobase peaks. Similar linear gradient of 0.9–10% of acetonitrile over 25 min was applied with a reduced flow rate of 0.8 mL/min. The eluted products were monitored at 293 nm.

Cumputational Details. Quantum chemical calculations were done in water using density functional theory (DFT) BHandHLYP methods[33,34] with 6-31+G(d,p) basis set (Jaguar version 7.6 program[35]) level of theory. The interactions between the molecule and the solvent were evaluated at the same level of theory by Jaguar's Poisson–Boltzmann solver (PBF)[36] which fits the field produced by the solvent dielectric continuum to another set of point charges. The frequency analysis was made on the structures optimized in water at the same level of theory to characterize the stationary points on the potential surface and to obtain thermodynamic parameters such as zero point energy (ZPE), entropy (S), and Gibbs free

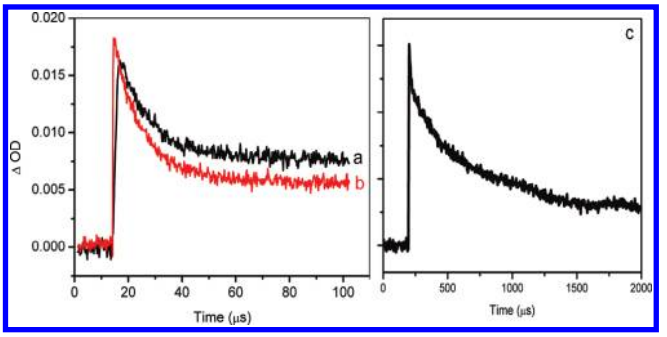


Figure 2. Transient absorption at 330 nm observed for the reaction of $^{\bullet}OH$ with guanine in N_2O-saturated solution (a and b) at 100 μs, pH 4.6 and 3.8, respectively, and (c) at 2000 μs after the pulse at pH 4.6. dose/pulse = 3−4 Gy/pulse.

energy (G) at 298 K. The reaction enthalpies (ΔH) and Gibbs free energies of reaction (ΔG) were calculated as the difference of the electronic energies ΔE_0 ($E_0 = E + ZPE$) and Gibbs free energies between the reactants and products respectively.

RESULTS AND DISCUSSION

The time-resolved transient spectral changes observed from the pulse irradiation of N_2O-saturated aqueous solution (0.5×10^{-3} mol dm^{-3}) of guanine (**I**, Scheme 1) at pH 4.6 are depicted in Figure 1. The absorption spectra recorded at 4 μs after the pulse showed transient absorption bands centered around 300 and 330 nm. The transient absorption band corresponding to 300 nm did not show any apparent decay within 20 μs, Figure 1 inset (a). However, there is an appreciable change in the transient absorption band corresponding to 330 nm (Figure 2, inset a). The decrease in the intensity of the transient absorption peak at 330 nm is accompanied by a build up at 525 nm. Further, the absorption decayed nearly 70% within 800 μs (Figure 1). This transient absorption spectrum is different from that reported[10,11,13,19] in the $^{\bullet}OH$ reaction with 2′-deoxyguanosine and guanosine derivatives. The spectral differences were also seen by us in the $^{\bullet}OH$ reaction with 1×10^{-3} mol dm^{-3} guanosine at pH's 6.7 and 4.4. The time-resolved spectra for both the pH's are similar (Figure 1S) and are similar with the reported transient absorption spectra[19] and the 330 nm transient absorption peak observed in case of $^{\bullet}OH$ reaction with guanine are absent. The traces recorded at 610 nm are also shown (insets a and b Figure 1S). The biphasic nature at pH 4.4 suggest an acid catalyzed water elimination reaction and/or water assisted tautomerization reaction as reported[13,19] which becomes slower near neutral pH.

When the rate of absorption growth at 330 nm (Figure 1, inset b) as a function of concentration of the solute at pH 4.6 is measured, the $^{\bullet}OH$ radical was found to react with guanine at diffusion-controlled rate ($\geq 1 \times 10^{10}$ mol^{-1} dm^3 s^{-1}). The second order rate constant is consistent with the reported[21] value (9.2×10^9 mol^{-1} dm^3 s^{-1}) for the $^{\bullet}OH$ reaction with guanine at pH 10 and to that of guanosine and 2′-deoxyguanosine derivatives.[10,11,13,19] The decay of the transient species formed by the reaction of $^{\bullet}OH$ with guanine at 300 and 330 nm was monitored. The trace at 300 nm (Figure 1, inset a) exhibited a slow build-up after the initial fast reaction of the $^{\bullet}OH$ and is similar to the ring-opening of the OH-adduct ($\lambda_{max.}$ = 300 nm) proposed for the reaction of $^{\bullet}OH$ with 2′-deoxyguanosine.[13] In contrast, the absorption at 330 nm (Figure 2a) underwent a fast

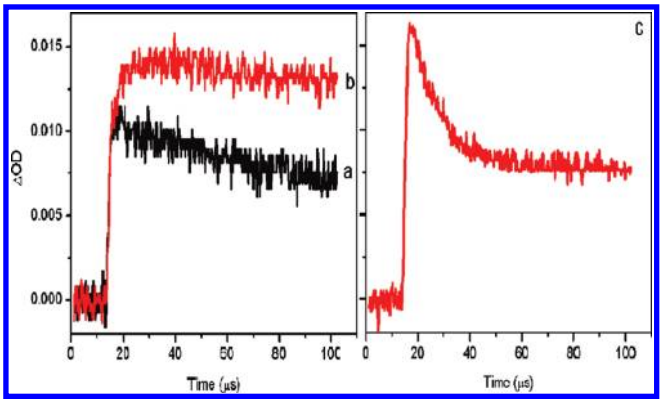


Figure 3. Change in the transient optical absorption observed in the reaction of $^{\bullet}OH$ with guanine in aqueous solution saturated with (a) N_2O:O_2 or (b) N_2O at 300 nm and (c) N_2O:O_2 at 330 nm; pH 4.6; dose/pulse = 3−4 Gy.

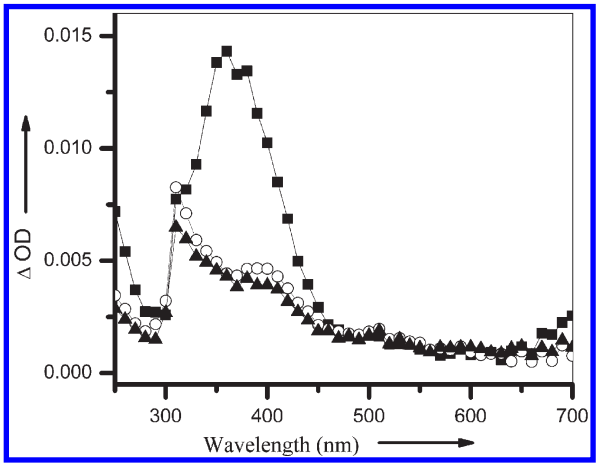


Figure 4. Time-resolved transient optical absorption spectra obtained from the pulse radiolysis of N_2O-saturated solution containing 0.5×10^{-3} mol dm^{-3} guanine and 0.1 mol dm^{-3} of potassium bromide at pH 4.6 recorded at 1 (■), 50 (○), and 200 μs (▲) after the pulse dose/pulse = 3−4 Gy.

decay with a first order rate constant $k = 4.5 \times 10^4$ s^{-1}, which is 1 order of magnitude higher than the reported water elimination reaction rate[13] for 2′-deoxyguanosine OH-adduct monitored at 620 nm. Furthermore, the decay rate constant was also found to be pH dependent with $k = 7 \times 10^4$ s^{-1} at pH 3.8 (Figure 2b). On a longer time scale, the transient species at 330 nm decayed by the usual bimolecular processes possibly to nonradical products (Figure 2c).

The characterization of the transient species absorbing at 300 and 330 nm was carried out by saturating the guanine solution with 4:1 (v:v) mixture of N_2O and O_2 to examine the oxygen effect. Its effect was clearly seen with the quenching of the slow build up at 300 nm observed in the absence of oxygen (Figure 3, panels a and b) whereas the peak at 330 nm remained unaffected (Figures 2a and 3c). The quenching of 300 nm absorption revealed its reaction with oxygen, and the species are reducing in nature.

The similarity in the decay of the transient species at 330 nm in the presence and absence of oxygen (Figure 2a and Figure 3c) indicates the lack of O_2 effect. This was further confirmed by the

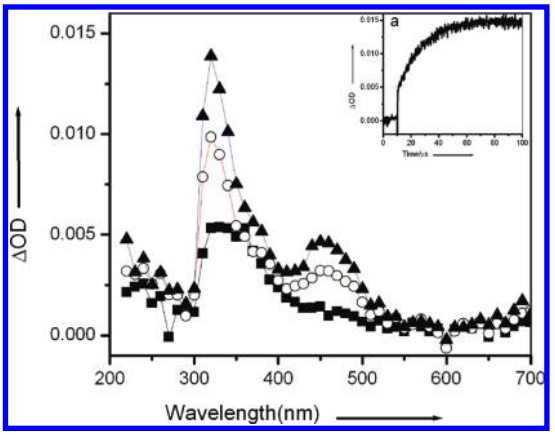


Figure 5. Time-resolved transient optical absorption spectra obtained from the pulse radiolysis of N_2O-saturated aqueous solution containing 0.5×10^{-3} mol dm^{-3} guanine and 0.1 mol dm^{-3} 2-propanol at pH 4.6 recorded at 2 (■), 16 (○), and 80 μs (▲) after the pulse dose/pulse = 3−4 Gy. Inset: Absorption at (a) 330 nm.

identical first order rate constant ($\sim 5.0 \times 10^4$ s^{-1}) observed with and without O_2 suggesting the oxidizing nature of the intermediate species.

The time-resolved transient spectral changes observed from the pulsed irradiation of N_2O-saturated aqueous solution of 0.5×10^{-3} mol dm^{-3} guanine (**I**, Scheme 1) containing 0.1 mol dm^{-3} KBr at pH 4.6 are depicted in Figure 4. The absorption spectra recorded at 1 μs show the absorption band at 360 nm similar to that of $Br_2^{\bullet-}$ reported[30] in the literature (Figure 2S) which decayed at longer time scale without any further spectral changes.

The transient spectra for the reaction of $Br_2^{\bullet-}$ with **I**, at 50 μs after the pulse is shown in Figure 4. The spectra exhibited transient absorption bands at 305, 400, and 510 nm and is different from the spectrum of $Br_2^{\bullet-}$ only (Figure 2S) indicating that $Br_2^{\bullet-}$ reacts with guanine, and its spectral features are identical to those measured for the $^{\bullet}$OH reaction with guanine at 20 μs after the pulse (Figure 1). Similarly, the spectrum for the reaction of $O_2^{\bullet-}$ with guanine at pH 4.6 exhibited identical absorption bands as measured in the $Br_2^{\bullet-}$ reaction but the absorption intensities are lower (Figure 3S). The slow reactivity of $O_2^{\bullet-}$ is possibly due to its low redox potential[37] (1.0 V) comparable to the redox potential of guanine (0.8−0.9 V in acidic pH). The obtained spectral features are also identical with the reported spectra for one-electron oxidized species of guanosine and 2′-deoxyguanosine derivatives.[38]

Further, the time-resolved transient spectra for the reaction of 2-hydroxy-2-propyl radical with guanine at pH 4.6 were also investigated. The transient absorption spectrum recorded at 2 μs exhibited a peak at 325 nm with a shoulder at 450 nm whose intensities increased with time (Figure 5). The formation trace at 340 nm shows an initial fast reaction followed by a slow build-up up to 100 μs (inset a, Figure 5). The species were found to be relatively stable with no appreciable decay even on a 2 ms time scale. The transient absorption spectra are nearly identical to that reported for guanine nucleosides,[16a,39,40] suggesting the formation of protonated electron adducts of guanine.

The HPLC chromatogram of the eluted nonradical products formed in the reaction of $^{\bullet}$OH with guanine at pH 4.6 is shown in Figure 6. The unirradiated guanine exhibits a single peak with retention time at 4 min (Figure 6a) and the parent peak is accompanied by two new peaks after the γ-irradiation of guanine aqueous solution purged with N_2O with an absorbed dose of 500 Gy

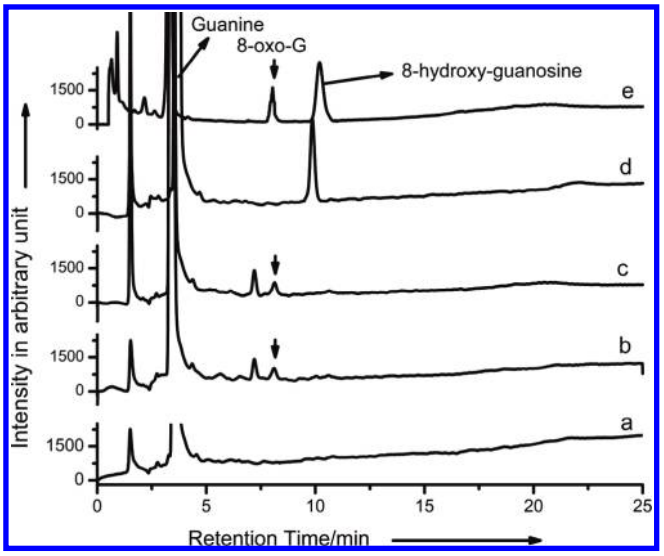


Figure 6. HPLC chromatogram of (a) γ-irradiated N_2O-saturated aqueous solution of 0.5×10^{-3} a mol dm^{-3} guanine at 0, (b) 500, (c) 500 Gy, with 1×10^{-4} mol dm^{-3} potassium ferricyanide, (d) mixture of guanine and 8-hydroxy-guanosine, unhydrolysed, and (e) acid-hydrolyzed 8-hydroxy-guanosine.

(Figure 6b, retention times 7 and 8 min) The intensities of the two product peaks eluted were enhanced on γ-irradiation of guanine aqueous solution in the presence of 1×10^{-4} mol dm^{-3} potassium ferricyanide (Figure 6c).

In order to examine the formation of 8-oxo-G, the procedure reported[41,42] earlier in irradiated DNA was employed. Standard 8-hydroxy-guanosine was acid hydrolyzed to cleave the glycosidic bond between the 8-oxo-G and the ribose sugar thereby liberating free 8-oxo-G. The HPLC chromatogram for the mixture of guanine and 8-hydroxy-guanosine is shown in Figure 6d, and the peak with the retention time 10 min corresponds to 8-hydroxy-guanosine. The acid hydrolyzed 8-hydroxy-guanosine shows an additional new peak eluting at 8 min along with the unhydrolysed 8-hydroxy-guanosine peak at 10 min (Figure 6e). This corresponds to one of the peaks of γ-irradiated aqueous solution of guanine with and without potassium ferricyanide and arises due to the formation of 8-oxo-G.

Energetics of OH Radical Reactions with Guanine and 2′-Deoxyguanosine. Quantum chemical calculations were performed to obtain the energetic of the $^{\bullet}$OH reaction with guanine and 2′-deoxyguanosine to complement the experimental data and the latter compound was chosen because theoretical calculations on guanosine have been reported[19,43,44] earlier. The molecular orbitals scheme and electron distribution in both guanine and 2′-deoxyguanosine are shown in Figure 7a and b respectively. It can be seen from the highest occupied molecular orbital (HOMO) (Figure 7a and b) that both guanine and 2′-deoxyguanosine show very similar electron distribution. The thermodynamic parameters, ΔH and ΔG for the two reaction pathways of $^{\bullet}$OH radical reaction with **I**; that is, addition to C4, C5, and C8 and the H-abstraction from N1, N2, and N9 positions are given in Scheme 2, parts a and b, respectively. Data obtained in the case of 2′-deoxyguanosine are shown in Scheme 1S, and our present results are in good agreement with those obtained on guanosine derivatives using B3LYP functional in water (dielectric continuum model SCRF = COSMO, Gaussian 03 program).[43,44]

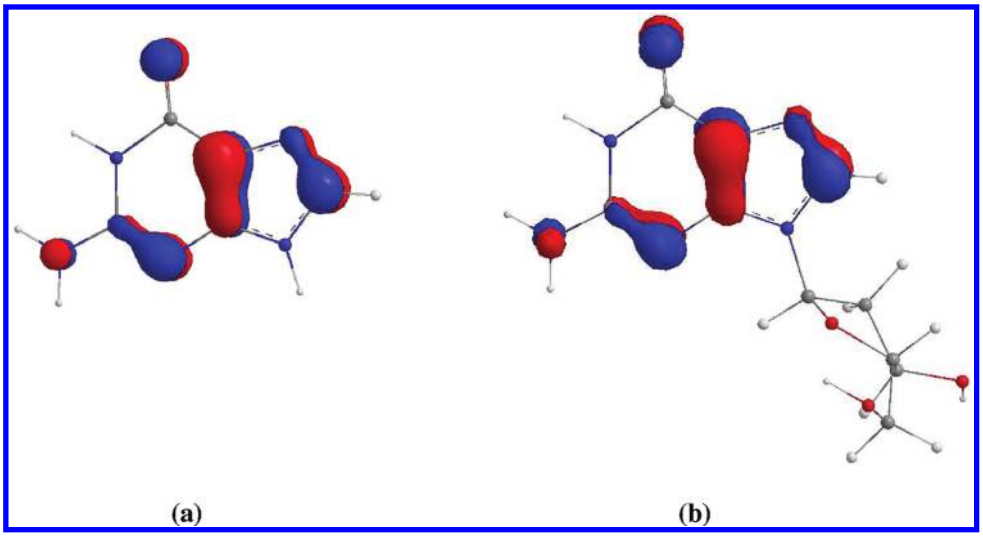


Figure 7. Electron distribution from highest occupied molecular orbitals (HOMO): (a) guanine and (b) 2′-deoxyguanosine.

The $^{\bullet}$OH addition on guanine results in the formation of three primary radicals C4-OH (**II**), the (reducing) C8-OH (**III**), and C5-OH (**VII**) adducts as depicted in Scheme 2a. The reaction parameters (ΔH and ΔG) show that the primary radicals C4-OH and C8-OH are energetically more favorable than the C5-OH. The C4-OH adduct (**II**) can undergo energetically favorable water elimination reaction to generate the oxidizing radical **V.** Further, it should be noted that the distribution of unpaired electron (spin) in the case of the C8-OH adduct is on N7 and not on C4 (Figure 4S).

Similarly, the $^{\bullet}$OH can abstract a H from **I** giving rise to three possible primary radicals- the aminyl radical (**IV**), N1-radical (**IX**), and N9-radical (**X**) (Scheme 2b). All three reaction pathways are energetically favorable. However, the H-abstraction from N9- of guanine leading to the generation of **X** is likely to be predominant. The radical **IV** can undergo water assisted tautomerization reaction leading to the formation of **V** and/or **X**, but the formation of the latter is energetically more favorable. However, our calculations clearly show that the tautomerization of radical **X** to the radical **V** is not a favorable process. Further, the guanyl radical **V** is relatively more stable than the radical **IV** with the ΔG being greater by 10 kJ mol^{-1} in the case of guanine (Scheme 2b). This agrees well with the preferred site of deprotonation at N1 in water over N2 (9 kJ mol^{-1}) as was obtained by us using the B3LYP/COSMO model[43] and by Adhikary et al.[45] (13.6 kJ mol^{-1}) who simulated the effect of the solvent by including seven water molecules in their calculations. This effect is even more pronounced (18 kJ mol^{-1}) in the case of 2′-deoxyguanosine (Scheme 1S).

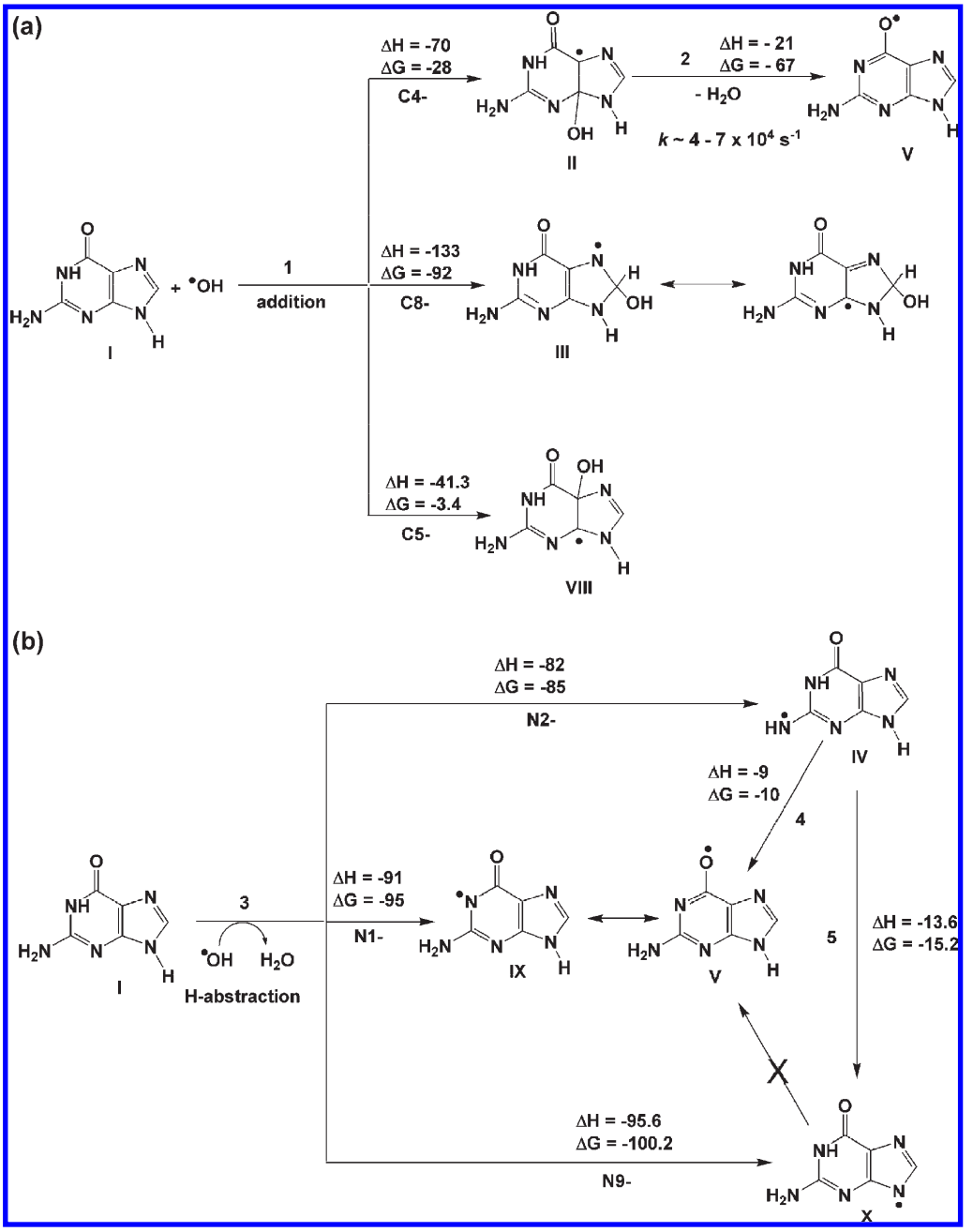
Computed Spectra of Various Radicals of Guanine. The question that needs to be addressed is whether the precursor for the radical **V** is the radical **II**, **IV**, or **X** (Schemes 2a and b). In other words, which of the two processes, addition of the $^{\bullet}$OH to C4 (reaction 1, Scheme 2a) followed by the dehydration (reaction 2) or the H abstraction (reaction 3, Scheme 2b) and the subsequent tautomerization (reaction 4) of the N-centered radical to the O-centered radical, is predominant.

In order to gain insight into the transient species involved in the $^{\bullet}$OH reaction with guanine, electronic transition spectra were calculated in water with the structure of guanine optimized in water geometry (SCRF = CPCM[43,44] model) using the unrestricted time dependent (UTD DFT) method at B3LYP/6-31+G(d) level as implemented in (Gaussian 03 package).[46] The time dependent (TDDFT) calculations of the UV−vis spectra for the various intermediate radicals formed by addition and H-abstraction by $^{\bullet}$OH were done at the optimized structures in water geometry at the BHandHLYP/6-31+G(d,p)/(SCRF = CPCM) level of theory, as implemented in Gaussian 03 program.[43,44] The radicals are denoted as G$^{\bullet+}$ for the guanine radical cation, C4-OH and C5-OH for the $^{\bullet}$OH addition product at the C4 and C5 positions of guanine, G(-H)1, G(-H)2, and G(-H)9 radicals formed from H abstraction at N1, N2, and N9 positions respectively and Table 1 lists their absorption peaks above 300 nm having significant intensities (oscillator strength ∼0.01). The computed spectral data are shown in Tables 1S−6S.

As can be seen, the C4-OH adduct spectrum did not show any absorption peak in the visible wavelength region in contrast to the other five radicals. For example, the guanine radical cation has a maxima peak at 335 nm followed by additional peak at 423 nm, the C5-OH adduct has a major peak at 417 nm accompanied by a smaller one at 306 nm. Further, the G(-H)1 and G(-H)9 radicals were found to have strong peaks 319 and 354 nm accompanied by less intense peaks at 411 and 558 nm respectively. The G(-H)2 radical has an intense peak at 504 nm and a smaller one at 360 nm. The higher wavelength values in the visible region reported by Chatgilialoglu and co-workers[19] and Galano and Alvarez-Idaboy[20] are red-shifted (600−650 nm). It should be, however, pointed out that our calculations were done without any restrictions in the visible region and the deviation is not large (0.5 eV between 450 and 550 nm) considering that the calculations are sensitive in the higher wavelength range.

Reaction Mechanism. The initial transient spectral characteristics of $^{\bullet}$OH reaction with guanine were found to be different from those reported with guanosine, 2′-deoxyguanosine and derivatives[10,11,13,19] with predominant peak at 330 nm and its first order fast decay. The H abstraction at N9- position could be a possibility for this observed difference. The unimolecular decay process seen in this work and the earlier reported for 2′-deoxyguanosine at low doses (1−4 Gy/pulse) could have been possibly suppressed with the overlapping bimolecular processes at high dose rates (ca. 22 Gy/pulse) employed in the recent work.[19] For example, the trace at 330 nm for the $^{\bullet}$OH reaction with guanine recorded with high dose rate ∼18 Gy/pulse at

Scheme 2. Calculated Reaction Parameters for the Reaction Pathways in the Case of Guanine: (a) Hydroxyl Radical Addition and (b) Hydrogen Abstraction



pH 4.6 is shown in Figure 5S. This trace is different from that observed at low dose rate (Figure 2a).

Based on the reactivity of intermediate radicals with oxygen, it is proposed that the transient species absorbing at 300 nm are assigned predominantly to the reducing C8-OH adduct. This is also supported by the TDDFT computed spectral data. The peak at 330 nm is attributed to the oxidizing species which is consistent with the mechanism already reported.[13] Further, the diffusion-controlled second order rate constant for the •OH reaction with guanine (>1 $\times 10^{10}$ mol^{-1} dm^3 s^{-1}) suggests that the •OH reacts mainly *via* addition mechanism as compared to the electron transfer and H-abstraction reactions whose second order rates constant are expected to be lower.

The quantum chemical calculations predict that the H-abstraction from the N9- position leading to the G(-H)9 radical **X** (Scheme 2b) is indeed an energetically favored pathway (Scheme 2b) and its spectrum with peaks at 333, 355, and 558 nm (Table 1 and 6S), is different from the observed initial spectrum for the •OH reaction with **I** (Figure 1). Similarly, except for the C4-OH adduct, the computed spectra are different from the experimental spectrum especially with peaks in the visible region. Therefore, though the radical **X** is predicted to be energetically favorable for the •OH reaction with guanine, the kinetics of the •OH reaction, the differences in the experimental and theoretical spectra and the energetics suggest that the radical **X** is a less likely precursor and the most probable is the C4-OH adduct (**II**, Scheme 2a) for the formation of the eventually stable radical **V**.

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Table 1. Computed Spectral Peaks (λ) of Various Guanine Radicals[a]

radical	absorption peaks/nm
Guanine radical cation $G^{\bullet+}$	335 (0.170)
	375 (0.023)
	423 (0.058)
C4-$^{\bullet}$OH adduct	301 (0.063)
	304 (0.070)
	328 (0.100)
C5-$^{\bullet}$OH adduct	306 (0.064)
	338 (0.067)
	417 (0.084)
$G^{\bullet}$(-H)1	319 (0.125)
	411 (0.075)
	540 (0.022)
$G^{\bullet}$(-H)2	361 (0.010)
	504 (0.194)
$G^{\bullet}$(-H)9	333 (0.098)
	354 (0.137)
	558 (0.012)

[a] Oscillator strength (f) is given in parentheses. The values of $\lambda > 300$ nm and $f \geq 0.01$ are only considered.

Both the one-electron oxidizing radicals, $Br_2^{\bullet-}$ and $O_2^{\bullet-}$ oxidized guanine giving rise to the guanyl radical cation (**VI**, Scheme 1). Water assisted deprotonation of guanyl radical cation for 2′-deoxyguanosine is known and the pK_a is reported to be 3.9.[47] However, due to lack of solubility of guanine above pH 4.8, the pK_a could not be determined for guanine. Therefore, the resulting spectra for the reaction of $Br_2^{\bullet-}$ and $O_2^{\bullet-}$ with guanine at pH 4.6 recorded after 50 and 1 μs respectively (Figures 4 and 3S) are assigned to the guanyl radical (**V**, Scheme 2a and b). Interestingly, the observed spectrum is identical to that obtained in the $^{\bullet}$OH reaction with guanine at pH 4.6 recorder at 20 μs (Figure 1) suggesting that the initial transient species decays to the one-electron oxidized guanyl radical (**V**, Scheme 2a).

The absorption spectra obtained in the reaction of 2-hydroxy-2-propyl radical with guanine and the traces recorded at 325 nm (Figure 5) suggest that the transient species generated by the $^{\bullet}$OH reaction with guanine are different from that of the protonated electron adducts of guanine by the 2-hydroxy-2-propyl radical. The reduction mechanism for guanosine, deoxyguanosine and derivatives has been established explicitely[16a,39,40] and leads to the formation of the protonated C8−N centered guanyl radical. The same mechanism is proposed for the reduction of guanine.

The product analysis using HPLC for the reaction $^{\bullet}$OH with guanine at pH 4.6 indicates that one of the nonradical products from the decay of the OH-adducts of guanine is 8-oxo-G eluting at 8 min (Figure 6b). The precursor for the formation of 8-oxo-G is known to be the C8-OH adducts of guanine (**III**, Scheme 2). This observation complements the formation of C8-OH adducts as one of the transient species produced in the $^{\bullet}$OH reaction with guanine at pH 4.6.

CONCLUSIONS

Both radiation and quantum chemical methods have been employed to address in detail, the mechanism of the reaction of oxidizing radicals ($^{\bullet}$OH and $Br_2^{\bullet-}$) with the nucleobase guanine. The addition of $^{\bullet}$OH leading to the initial formation of C4-OH (330 nm) and C8-OH (300 nm) adducts is the preferred reaction pathway. Subsequently the former radical eliminates water resulting in the formation of the guanyl radical which is also generated by $Br_2^{\bullet-}$ in its reaction with guanine. The spectra of various intermediate radicals were computed and the assignment of the C4-OH adduct peak is in good agreement with the computed spectrum, energetics and its lack of reactivity toward molecular oxygen. Further, the tautomerization of the radical **X** formed by H-abstraction from N9- is not likely. The formation of 8-oxo-G was seen from the precursor C8-OH adduct under steady state radiolysis. The combination of radiation chemical methods, both pulse and steady state radiolysis, with quantum chemical studies forms an excellent tool in the understanding of the radical reaction mechanisms.

ASSOCIATED CONTENT

Supporting Information. (i) Transient absorption spectra obtained in the $^{\bullet}$OH radical reaction with guanosine (Figure 1S). (ii) Transient optical absorption spectra obtained in the $Br_2^{\bullet-}$ radical reaction with guanine (Figure 2S). (iii) Transient absorption spectra obtained in the $O_2^{\bullet-}$ radical reaction with guanine (Figure 3S). (iv) Calculated reaction parameters for the reaction pathways in the case of 2′-deoxyguanosine (Scheme 1S, Figure 4S). (v) Traces obtained at 330 nm in the reaction of $^{\bullet}$OH with guanine at a dose rate of 18 Gy/pulse; pH 4.6 (Figure 5S). (vi) Computed spectral data of intermediate radicals formed with guanine (Tables 1S−6S). This material is available free of charge via Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: geetas@chem.unipune.ac.in. Phone: +91 20 25696062, ext. 553. Fax: +91 20 25691728.

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