Hydroxyl radical-induced degradation of 2′-deoxyguanosine under reducing conditions

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Addition of hydroxyl radical to the base moiety of 2′-deoxyguanosine (dGuo) leads to the formation of two main radicals exhibiting oxidising and reducing properties, respectively. The oxidising radical reacts with oxygen to yield 2,2-diamino-5-[2-deoxy-β-D-*erythro*-pentofuranosyl)amino]oxazol-5(2*H*)-one (oxazolone) as the final product. The reducing radical is either preferentially oxidised into 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) or reduced into a 2,6-diamino-4-hydroxy-5-formamidopyrimidine derivative (FapydGuo) depending on conditions. We report here that the presence of reducing compounds (ascorbate or cysteine) strongly modifies the distribution of modified nucleosides upon γ irradiation of an aerated aqueous solution of dGuo. The yield of oxazolone decreases while that of 8-oxodGuo and FapydGuo increases. This was explained by the reduction of the oxidising radical which prevents the occurrence of the restitution of dGuo through a reaction between the oxidising and the reducing purine radicals. The study was extended to the decomposition of dGuo upon photochemical release of ˙OH by *N*-hydroxypyrimidine-2-thione (HPT). The analysis of the base modification products of dGuo induced by the latter system showed that HPT exhibits reducing properties and cannot be used as a pure photochemical source of ˙OH radical.

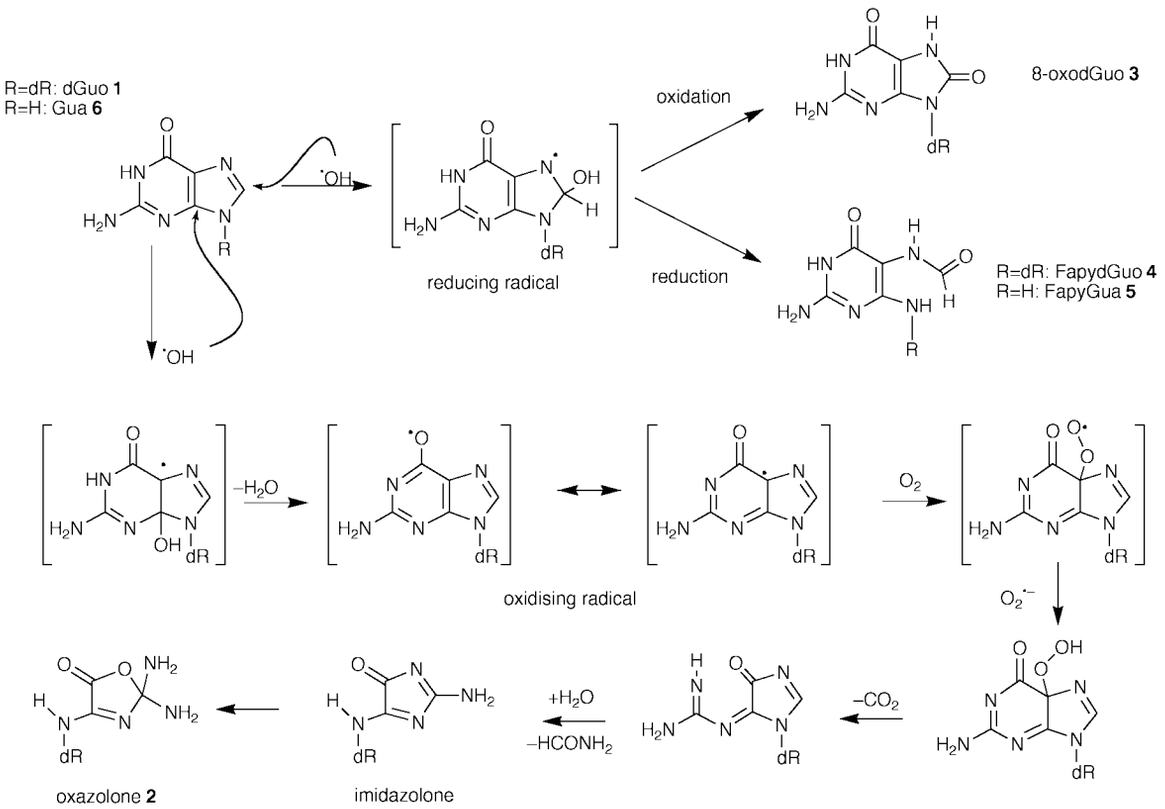
Introduction

Damage to cellular DNA is a major deleterious event which may lead to cell death and the appearance of mutations. Hydroxyl radical (˙OH), produced either by radiation-induced decomposition of water or Fenton chemistry, is among the most DNA damaging species. Degradation of DNA by ˙OH radicals produces a wide range of lesions, including strand-breaks, DNA–protein crosslinks, abasic sites and base modifications.[1] The bulk of initial radicals produced by the addition of ˙OH to DNA bases have been identified by EPR and pulse radiolysis studies.[2–4] In addition, the main final ˙OH-mediated degradation products of the four DNA bases have been isolated and characterized mainly as the results of steady-state gamma radiolysis experiments carried out on nucleosides.[5] Comprehensive mechanisms which can account for the formation of the latter modified nucleosides are now available. Two main pathways have been proposed for the hydroxyl radical induced-degradation of 2′-deoxyguanosine **1** (dGuo). Indeed, two main radicals are produced depending on the site of addition of ˙OH to the purine ring (Scheme 1). The C4 adduct dehydrates into an oxidising oxyl radical which further reacts, probably as a C5 centred mesomeric form, with oxygen to yield an imidazolone derivative after subsequent rearrangement. The latter compound undergoes a hydrolytic conversion into 2,2-diamino-4-[2-deoxy-β-D-*erythro*-pentofuranosyl)amino]oxazol-5(2*H*)-one **2** (oxazolone).[6,7] Addition of ˙OH to the C8 position of dGuo gives rise to a reducing radical. The latter intermediate can be either oxidised into 8-oxo-7,8-dihydro-2′-deoxyguanosine **3** (8-oxodGuo) or reduced into 2,6-diamino-4-hydroxy-5-formamidopyrimidine nucleoside **4** (FapydGuo). This is in agreement with observations made within isolated DNA upon exposure to ˙OH radicals in aqueous solution. Indeed, 8-oxodGuo **3** is the major degradation product within DNA exposed to γ-rays under aerobic conditions.[8] However, a drastic increase in the yield of FapydGuo **4** at the expense of 8-oxodGuo **3** is observed when oxygen is absent.[9] In contrast to what is observed in DNA, 8-oxodGuo **3** is generated only in a very low yield when an aerated aqueous solution of dGuo **1** is exposed to gamma radiation. In contrast, oxazolone **2** is produced with a high efficiency.[10] The present work provides evidence for the occurrence of an efficient reaction between the predominant oxidising and the reducing purine radicals leading to a restitution of the guanine moiety. This may account for the differences in the distribution of guanine degradation products observed upon steady-state radiolysis of aqueous solutions of nucleoside on one hand and DNA on the other hand. This was inferred from the study of the influence of added reducing agents on the distribution of the final products observed upon gamma irradiation of dGuo in aerated aqueous solution. The latter results have then been compared with those obtained by using the photolysis of *N*-hydroxypyridine-2-thione as a source of ˙OH.

Results and discussion

Radiation-induced degradation products of dGuo 1 in aerated aqueous solution

Three main base modification products, including oxazolone **2**, 8-oxodGuo **3** and FapydGuo **4**, were found to be generated in gamma-irradiated solutions of dGuo **1**. The level of 8-oxodGuo was measured by HPLC-electrochemical (EC) detection[11] while oxazolone and its imidazolone precursor were quantitated by an indirect HPLC-fluorescence assay.[12] The formamidopyrimidine derivative of dGuo was not analysed as a nucleoside since it rapidly interconverts into a mixture of α and β furanosidic and pyranosidic isomers due to the instability of its *N*-glycosidic bond.[13] Therefore, the formation of FapydGuo **4** was monitored by GC-MS following a mild formic acid hydrolysis at room temperature. This was used as an alternative to either the hot formic acid hydrolysis which leads to a degradation of the lesion or to the reliable but time consuming HF–pyridine-mediated release of the base (FapyGua **5**).[9] Free



Scheme 1 Formation of oxazolone **2**, 8-oxodGuo **3** and FapydGuo **4** upon $^{\bullet}OH$-induced degradation of dGuo **1** (dR: 2-deoxyribose).

guanine **6** was also quantitated by GC-MS and used as a marker of the radiation-induced damage to the sugar moiety of the nucleoside. Both GC-MS assays involved the use of isotopically labelled internal standards.

Gamma irradiation of dGuo in the presence of cysteine and ascorbate

Reactions of ascorbate and cysteine with $^{\bullet}OH$-induced guanine radicals have already been observed by pulse radiolysis.[2] In the present work, the effect of the presence of the two latter reducing compounds on the distribution of gamma radiation-induced base damage was studied. Both ascorbate and cysteine exhibit scavenging properties for $^{\bullet}OH$ with rate constants of 4×10^{9} [14–17] and 1.9×10^{10} L mol^{-1} s^{-1},[18] respectively. This must be compared with the rate constant for the reaction of $^{\bullet}OH$ with 2′-deoxyguanosine 5′-monophosphate which is 6.8×10^{9} L mol^{-1} s^{-1}.[19] It appears that a low concentration of reducing species must be used to prevent significant scavenging of $^{\bullet}OH$ and also to minimise the role of secondary radicals produced by the reaction of $^{\bullet}OH$ with either cysteine or ascorbate. Therefore, we studied the effects of the latter reducing compounds within a wide range of concentrations (0.01 to 1 mM) on the radiation-induced degradation of dGuo **1** (1 mM). As already reported, it was observed that gamma irradiation of an aerated aqueous solution of dGuo **1** led to the predominant formation of oxazolone[10] whereas FapydGuo **4** and 8-oxodGuo **3** were produced only in minute amounts. The yield of released guanine **6** was found to be at least 5 times lower than that of the overall base degradation products. This is in agreement with the estimation that $^{\bullet}OH$-induced hydrogen abstraction to the 2-deoxyribose moiety represents 10 to 20% of the overall yield of addition of $^{\bullet}OH$ to the purine ring.[2] When either cysteine or ascorbate was added, even at low concentration, a drastic decrease in the yield of oxazolone was observed along with an increase in the formation of 8-oxodGuo **3** and FapydGuo **4**. In contrast, the extent of guanine **6** release remained unchanged. The decrease in the yield of oxazolone **2** is consistent with the highly oxidising character of its oxyl radical precursor. Reduction of the latter species by both ascorbate and cysteine has already been observed in pulse-radiolysis studies.[2] Interestingly, similar results were obtained, irrespectively of the agent used. This shows that the "chemical repair" of guanine

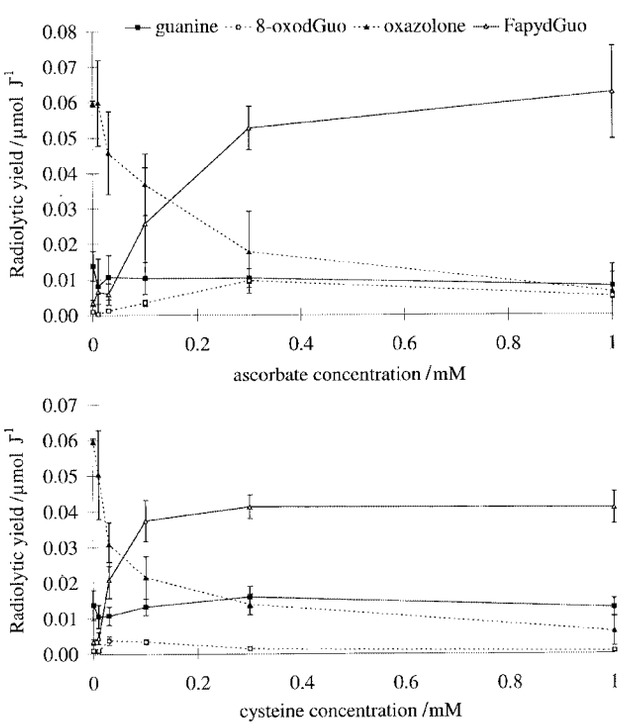


Fig. 1 Influence of the concentration of ascorbate (upper panel) and cysteine (lower panel) on the radiolytic yield (expressed in μmol J^{-1}) of formation of 8-oxodGuo **3**, FapydGuo **4**, oxazolone **2** and free guanine **5** upon exposure of dGuo **1** (1 mM) to gamma radiation in aerated aqueous solution.

Table 1 Proportion (expressed in %) of $^{\bullet}OH$ reacting with dGuo in the presence of various concentrations of ascorbate and cysteine. These values have been used as a correcting factor to determine the overall degradation yield of the guanine moiety reported in Fig. 2

Concentration/mM	Ascorbate	Cysteine
0	100	100
0.01	99	97
0.03	98	92
0.1	95	79
0.3	85	55
1	64	27

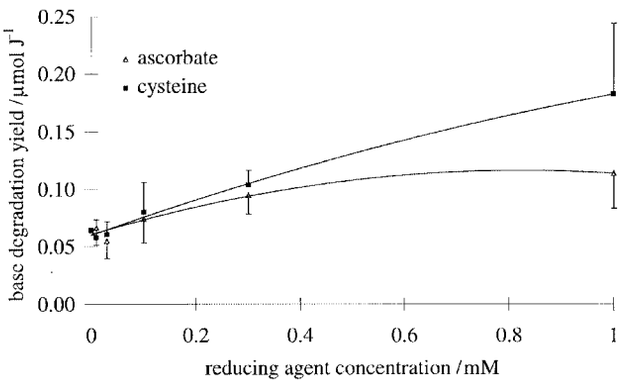


Fig. 2 Influence of the concentration of ascorbate and cysteine on the combined radiolytic yield (expressed in μmol J^{-1}) of oxazolone **2**, 8-oxodGuo **3** and FapydGuo **4** upon exposure of dGuo **1** (1 mM) to gamma radiation in aerated aqueous solution. The values were corrected from the scavenging of $OH^{\bullet}$ by the reducing species added to the sample.

radicals by cysteine does not necessarily involve H donation as is often proposed for thiol containing compounds. Indeed, the latter products may also behave as electron donating reducing species in a similar way to ascorbate.

Evidence for a restitution reaction of dGuo

The high increase, by about one order of magnitude, of the combined yield of 8-oxodGuo **3** and FapydGuo **4** with a 0.1 mM concentration of any of the reducing agents used is a striking observation. Indeed, the rate of formation of the initial reducing radical depends only on the steady-state concentration of $^{\bullet}OH$. However, the available amount of $^{\bullet}OH$ is expected to decrease when reactive compounds are added to the solution of dGuo **1**. Another interesting observation concerns the combined yield of the three degradation products of the base moiety of dGuo **1**. For this purpose, the hydroxyl radical scavenging properties of the added reducing compound had to be taken into account. Indeed, the relative amount of hydroxyl radicals involved in the reaction with dGuo **1** ($R_{^{\bullet}OH/dGuo}$) decreases as the concentration in reducing species increases. $R_{^{\bullet}OH/dGuo}$ can easily be calculated (Table 1) from the concentration and the reaction rate constants with $^{\bullet}OH$ of dGuo **1**, ascorbate and cysteine by using eqn. (1).

$$R_{^{\bullet}OH/dGuo} = k_{dGuo/^{\bullet}OH} \times [dGuo]/(k_{red/^{\bullet}OH} \times [red] + k_{dGuo/^{\bullet}OH} \times [dGuo]) \quad (1)$$

Using this correcting factor, the combined yield of 8-oxodGuo **3**, FapydGuo **4** and oxazolone **2** was found to increase with increasing concentrations of reducing species (Fig. 2). This observation together with the increase in the yield of FapydGuo **4** and 8-oxodGuo **3** can be rationalised in terms of the occurrence of a reaction between the reducing and oxidising radicals of guanine leading to the restitution of dGuo **1**. Increase in the yield of 8-oxodGuo upon exposure of dGuo to gamma rays in the presence of thiols and ascorbate has recently been reported.[20] The authors proposed the involvement of secondary radicals. However, no other base damage was measured and the balance between the products arising from the reducing and the oxidising radicals could not be observed. Pulse radiolysis studies have shown that the yield of the oxidising radical is twice that of the reducing radical.[2] The occurrence of an efficient reaction between the two latter radicals is expected to result in the significant consumption of the reducing radical. This could therefore account for the observation that oxazolone is the major base modification product formed upon irradiation of dGuo **1** in pure water. When ascorbate and cysteine are added, the oxidising radical is partially reduced to dGuo **1**. This explains the decrease in the yield of oxazolone **2**. In addition, the decrease in the steady-state concentration of oxidising radical leads to a decrease in the yield of its reaction with the reducing radical. This is expected to lead to an increase in the steady-state level of reducing radical. This accounts for the increase in the yield of FapydGuo **4** and 8-oxodGuo **3** with increasing concentrations of reducing species. Further indication for a reaction between the oxidising and reducing radicals of dGuo **1** was provided by the determination of the radiolytic degradation yield of nucleosides in aerated aqueous solution. The radiolytic degradation yield of thymidine (0.219 μmol J^{-1}) and 2′-deoxycytidine (0.211 μmol J^{-1}) is close to that of the formation of $^{\bullet}OH$ (0.25 μmol J^{-1}). In contrast, the radiolytic degradation yield of dGuo **1** is less than half of this value (0.101 μmol J^{-1}). The comparison between the latter values strongly supports the occurrence of a restitution reaction in the γ-radiolysis of dGuo **1**. It can be added that FapydGuo **4** is produced in higher yield than 8-oxodGuo **3** because FapydGuo **4** results from the reduction of the reducing radical whereas 8-oxodGuo **3** is produced by its oxidation. The only oxidant in the reaction mixture is oxygen. It can thus be concluded that the reducing radical reacts quite slowly with O_2, as inferred from the low yield of 8-oxodGuo **3**. This is in agreement with results obtained from time-resolved studies which showed the lack of quenching of the signal of guanine radicals by oxygen on the microsecond time scale ($k < 10^6$ L mol^{-1} s^{-1}).[2] It can be added that the reaction of the reducing radical with either cysteine or ascorbate is also quite slow. Indeed, the formation of FapydGuo **4** and 8-oxodGuo **3** is expressed in eqns. (2) and (3), where

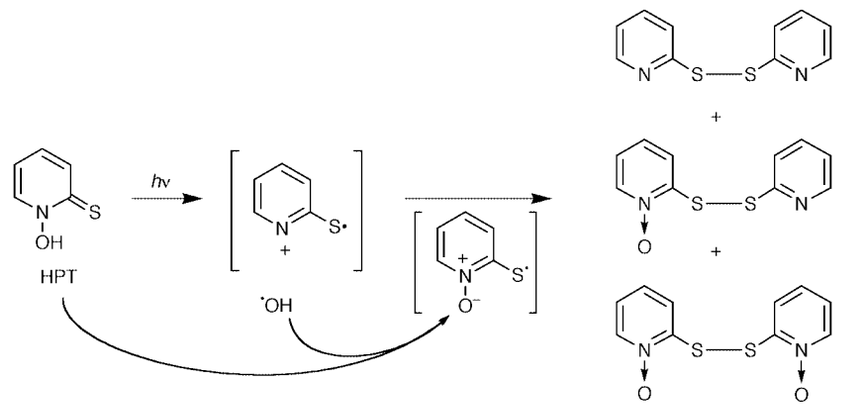
$$d[Fapy]/dt = k_{Rred/Red} \times [Rred] \times [Red] \quad (2)$$

$$d[8\text{-}oxodGuo]/dt = k_{Rred/O_2} \times [Rred] \times [O_2] \quad (3)$$

[Rred] is the steady-state concentration in reducing radical, [Red] the concentration in reducing compound (cysteine or ascorbate), $k_{Rred/Red}$ the reaction rate constant between the reducing radical and the reducing compound, and k_{Rred/O_2} the reaction rate constant between the reducing radical and oxygen. The ratio between the radiolytic yield (G) of FapydGuo **4** and 8-oxodGuo **3** is given in eqn. (4).

$$G_{FapydGuo}/G_{8\text{-}oxodGuo} = k_{Rred/Red} \times [Red]/k_{Rred/O_2} \times [O_2] \quad (4)$$

Based on the assumption that the oxygen concentration is 300 μM and that $k_{Rred/O_2} < 10^6$ L mol^{-1} s^{-1},[2] it can be calculated from the slope of the function $G_{FapydGuo}/G_{8\text{-}oxodGuo}$ = f([Red]) that $k_{Rred/Red} < 1.6 \times 10^6$ L mol^{-1} s^{-1} for ascorbate and $k_{Rred/Red} < 1.3 \times 10^7$ L mol^{-1} s^{-1} for cysteine. The latter values suggest that the reduction of the 8-hydroxy-7,8-dihydroguanin-7-yl radical is a rather slow process. These results together with the low value of the rate constant for the reaction of guanine radicals with oxygen are likely to explain why radicals produced with steady-state concentration in the nanomolar range are able to react with each other. However, this implies that the restitution of dGuo **1** through the reaction between the reducing and the oxidising radicals is a diffusion controlled



Scheme 2 Mechanism of the photoinduced release of •OH from *N*-hydroxypyridine-2-thione (adapted from Figure 2 of B. Epe, D. Ballmaier, W. Adam, G. N. Grimm and C. R. Saha-Möller, *Nucleic Acids Res.*, 1996, **24**, 1625, by permission of Oxford University Press).

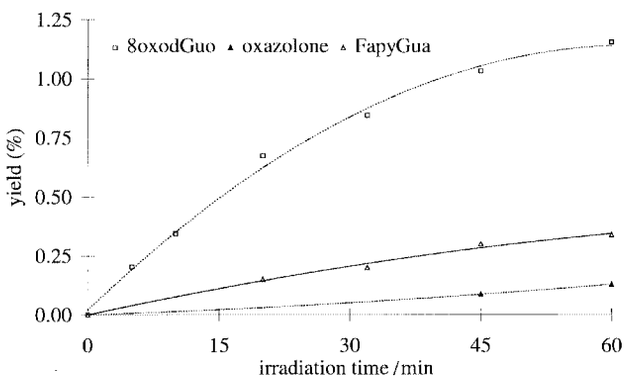


Fig. 3 Time course study of the formation of 8-oxodGuo **3**, FapydGuo **4** and oxazolone **2** in a 1 mM solution of dGuo upon photolysis of *N*-hydroxypyridine-2-thione (10 mM) by 300 nm light.

process. The restitution reaction is likely to account for a major part of the difference between the guanine radiation-induced damage distribution within DNA on one hand and dGuo on the other hand. Indeed, the restitution reaction cannot take place within DNA because the radicals are linked to the phosphodiester backbone and cannot diffuse. In addition, very high radiation dose rates would be required to produce either vicinal radicals or radicals close enough to each other to allow charge transfer processes to occur. Consequently, products arising from the 8-hydroxy-7,8-dihydroguanin-7-yl reducing radical are produced in significant yield within DNA, even in the absence of reducing compounds.

Degradation of dGuo 1 through the photolysis of *N*-hydroxypyridine-2-thione

The photolysis of *N*-hydroxypyridine-2-thione (HPT) has been proposed as a source of •OH radicals[21] (Scheme 2). Extensive studies of the photochemistry of HPT have recently shown that the photo-induced release of •OH leads to the formation of several degradation products of HPT which may exhibit photosensitization properties.[22–24] It should be added that substituted derivatives of HPT have been used as generators of alkoxy radicals.[25] The strategy applied for the determination of the effects of reducing agents on the radiation-induced decomposition of dGuo **1** was used in order to assess the reliability of HPT as a photochemical source of •OH. dGuo **1** was used as the substrate and the final decomposition products distribution was compared with that generated by gamma radiation. A time-course study of the formation of 8-oxodGuo **3**, FapydGuo **4** and oxazolone **2** upon HPT-mediated photolysis of dGuo **1** at 300 nm was carried out (Fig. 3). Interestingly, 8-oxodGuo **3** was found to be the main photo-induced dGuo decomposition product. The initial rate of formation of FapydGuo **4** and oxazolone **2** represented 17 and 6% of that of 8-oxodGuo **3**, respectively. These results are drastically different from the base damage distribution induced by exposure of dGuo **1** to gamma radiation in aerated aqueous solution (*vide supra*). Under the latter conditions, oxazolone **2** is the major product whereas 8-oxodGuo **3** and FapydGuo **4** are only produced in minor amounts. Degradation of dGuo **1** by photolysis of HPT mainly leads to products arising from the reducing radical. This observation is similar to that made upon steady-state gamma radiolysis of aqueous solutions of dGuo **1** in the presence of reducing compounds such as ascorbate and cysteine. Therefore, it may be suggested that HPT and/or its decomposition products exhibit reducing properties which modify the chemistry of the •OH-induced dGuo radicals. However, a main difference between gamma irradiation in the presence of cysteine or ascorbate and HPT-mediated photodegradation of dGuo is that the yield of FapydGuo **4** is lower than that of 8-oxodGuo **3** under the latter conditions.

Table 2 Radiolytic yields of formation of 8-oxodGuo **3**, FapydGuo **4** and oxazolone **2** under various irradiation conditions in aerated aqueous solution (expressed in μmol J^{-1})

Irradiation conditions	8-OxodGuo	FapydGuo	Oxazolone
HPT 1 mM	0.0128	0.0081	0.0025
Decomp. HPT 1 mM	0.0216	0.0137	0.0034
HPT 10 mM	0.0059	0.0075	<0.0005
Water	0.0010	0.0032	0.0600
Ascorbate 1 mM	0.0051	0.0614	0.0061
Cysteine 1 mM	0.0009	0.0421	0.0064

To confirm the reducing properties of HPT, dGuo **1** was exposed to gamma radiation in aerated solution in the presence of HPT (1 or 10 mM). The experiment was resumed with a solution of HPT (1 mM) which had first been decomposed by exposure to UV light. The radiolytic yields of formation of the three purine products were measured in the three solutions and compared to the values obtained in pure water or in the presence of either cysteine or ascorbate (Table 2). It was clearly observed that the yield of oxazolone **2** drastically decreased in the presence of HPT or its decomposition products. In contrast, the formation of 8-oxodGuo **3** and FapydGuo **4** was enhanced. As observed upon degradation of dGuo **1** by photolysis of HPT, 8-oxodGuo **3** is the major decomposition product. This may be rationalised in terms of the reducing features of HPT and its photolysis products which lead to a decrease in the yield of oxazolone **2** by reaction with the oxidising radical arising from the addition of •OH on the C4 position of guanine. As observed with cysteine and ascorbate, this gives rise to the formation of 8-oxodGuo **3** and FapydGuo **4** in high yields. However, in contrast to the results obtained in the presence of the two latter reducing species, the yield of 8-oxodGuo **3** is

higher than that of FapydGuo **4**. This may be accounted for by a lower ability of HPT than ascorbate or cysteine to reduce the 8-hydroxy-7,8-dihydroguanin-7-yl radical.

Experimental

Chemicals

2′-Deoxyguanosine (dGuo) was obtained from Pharma-Waldhof. L-Cysteine, ascorbic acid, *N*-hydroxypyridine-2-thione (HPT) and 1,2-naphthoquinone-4-sulfonic acid (NQS) were purchased from Sigma (St. Louis, MO). Analytical grade formic acid (99%) was obtained from Merck (Darmstadt, Germany).

Gamma irradiation and HPT-mediated photodegradation of dGuo

Fresh stock solutions of cysteine and ascorbic acid (10 mM, pH 7) were prepared before each series of irradiations. The required volume of the stock solutions of reducing agents and water were added to 2.7 mL of a 1 mM solution of dGuo (final volume 3 mL) through which air was bubbled for 1 h. The final concentrations in cysteine and ascorbate were 0, 0.01, 0.03, 0.1, 0.3 and 1 mM. Aqueous solutions of dGuo containing either 1 mM HPT, 10 mM HPT or 1 mM UVB-degraded HPT were also prepared. The samples were then exposed under constant air saturation to the gamma rays emitted by a ^{60}Co source. The dose rate was 20 Gy min^{-1}. Aliquot fractions (1 mL) were collected after 0, 15 and 30 min of irradiation. All experiments were duplicated with new solutions of all reagents. A 1 mM solution of dGuo containing 10 mM HPT was exposed for increasing periods of time to the 300 nm light emitted by a Rayonet photoreactor (The Southern New England Ultraviolet Company, Hamden, CT) equipped with ten 15 W lamps. Irradiation times were 0, 20, 32, 45 and 60 min. Air bubbling was maintained during the experiment.

Analysis of modified bases

8-OxodGuo **3** was measured by HPLC-EC. The analytical system consisted of a model 2150 LKB pump (Pharmacia LKB Biotechnology, Uppsala, Sweden) connected to a SIL-9A autosampler (Shimadzu, Kyoto, Japan) equipped with an Uptisphere ODSB (particle size 5 μm) octadecylsilyl silica gel column (250 × 4.6 mm id) (Interchim, Montluçon, France). The isocratic eluent was a 50 mM aqueous solution of potassium phosphate (pH 4.7) containing 8% methanol. Coulometric detection of 8-oxodGuo **3** was provided by a Coulochem II detector equipped with a 5010 cell (ESA, Chelmsford, MA) with the potentials of the two electrodes set at 200 and 400 mV. The retention time of 8-oxodGuo **3** was 17 min. Elution of dGuo was simultaneously monitored by a Waters 484 UV variable wavelength spectrophotometer set at 280 nm. Both EC and UV signals were collected on a D7500 Hitachi integrator (Tokyo, Japan). Oxazolone **2** and its imidazolone precursor were measured by HPLC-fluorescence following decomposition under alkaline conditions. Typically, the irradiated solution of dGuo **1** (50 μL) was placed in a polypropylene vial together with 50 μL of 1 M sodium hydroxide. After homogenization, the solution was held at 70 °C for 30 min in a water bath. Then, the sample was removed and cooled down to room temperature. An aqueous solution of NQS (10 μL, 8 mg mL^{-1}) was added and the resulting solution incubated for 30 min in a water bath at 37 °C. After cooling to room temperature, the sample was neutralised by addition of 50 μL of 1 M hydrochloric acid. The solution was then analysed with the HPLC system described previously,[12] using the fluorescence detection provided by a F-1050 fluorimeter (Hitachi, Tokyo, Japan) with the excitation and emission wavelengths set at 355 and 405 nm, respectively. The eluent was a [90 : 10] v/v mixture of a 25 mM aqueous solution of ammonium formate and methanol. The amount of NQS derivative of guanidine (R_t: 11.3 min) was inferred from a calibration curve established with derivatized authentic guanidine. FapydGuo **4** and free guanine **6** (Gua) were measured by GC-MS. An aliquot of the solution of dGuo **1** (50 μL) was dried under vacuum together with 10 μL of a 66 μM solution of [$^{15}N_3$]FapyGua. The resulting residue was solubilized in 150 μL of 88% formic acid. The samples were left 15 min at room temperature and then evaporated to dryness under vacuum. Water (50 μL) was added and the solution dried under vacuum. The latter step was resumed once. The dry residue was then silylated in 100 μL of a [1 : 1] mixture of *N*-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylsilyl chloride (BSTFA, Aldrich, Milwaukee, WI) and acetonitrile for 25 min at 110 °C. The resulting solution was evaporated to dryness and silylated. Samples were analysed on a GC-MS apparatus consisting of a HP 5890 chromatograph and a MSD 5972 mass detector used in the single ion monitoring mode. Samples (1 μL) were injected in the splitless mode onto an Optima-5 column (25 m; 0.2 mm id; 0.1 μm film thickness) from Macherey-Nagel (Düren, Germany). The temperature of the injector was 250 °C. The column was maintained at 110 °C for 1 min. Then, the temperature was linearly increased to 280 °C at a rate of 25 °C min^{-1}. The ions collected were the following: FapyGua (retention time 5.95 min): $m/z = 457$ (FapyGua + 4 TMS), 442 (FapyGua + 4 TMS − methyl), 460 ([$^{15}N_3$]FapyGua + 4 TMS), 445 ([$^{15}N_3$]FapyGua + 4 TMS − methyl). For the measurement of guanine, 9 μL of a 57 μM solution of [$^{15}N_3$,^{13}C]Gua was added to 50 μL of the dGuo solution. The sample was evaporated to dryness, silylated by BSTFA and analysed by GC-MS. The ions collected were (retention time 6.01 min): $m/z = 367$ (Gua + 3 TMS), 352 (Gua + 3 TMS − methyl), 371 ([$^{15}N_3$,^{13}C]Gua + 3 TMS), 356 ([$^{15}N_3$,^{13}C]Gua + 3 TMS − methyl).

Determination of the radiolytic degradation yield of the nucleosides

Aqueous solutions (5 mL, 1 mM) of either 2′-deoxycytidine, thymidine or dGuo were exposed to the γ-rays emitted by the ^{60}Co source under constant air bubbling. After increasing periods of time (0, 15, 30 and 60 min) aliquot fractions (0.5 mL) were collected. Each fraction was injected on the HPLC system described above. Content of nucleoside was inferred from the area of the peaks observed on the UV chromatogram. The degradation was found to be linear over the dose range studied. Radiolytic degradation yields were calculated from the slopes obtained by linear regression. Each experiment was done in triplicate.

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