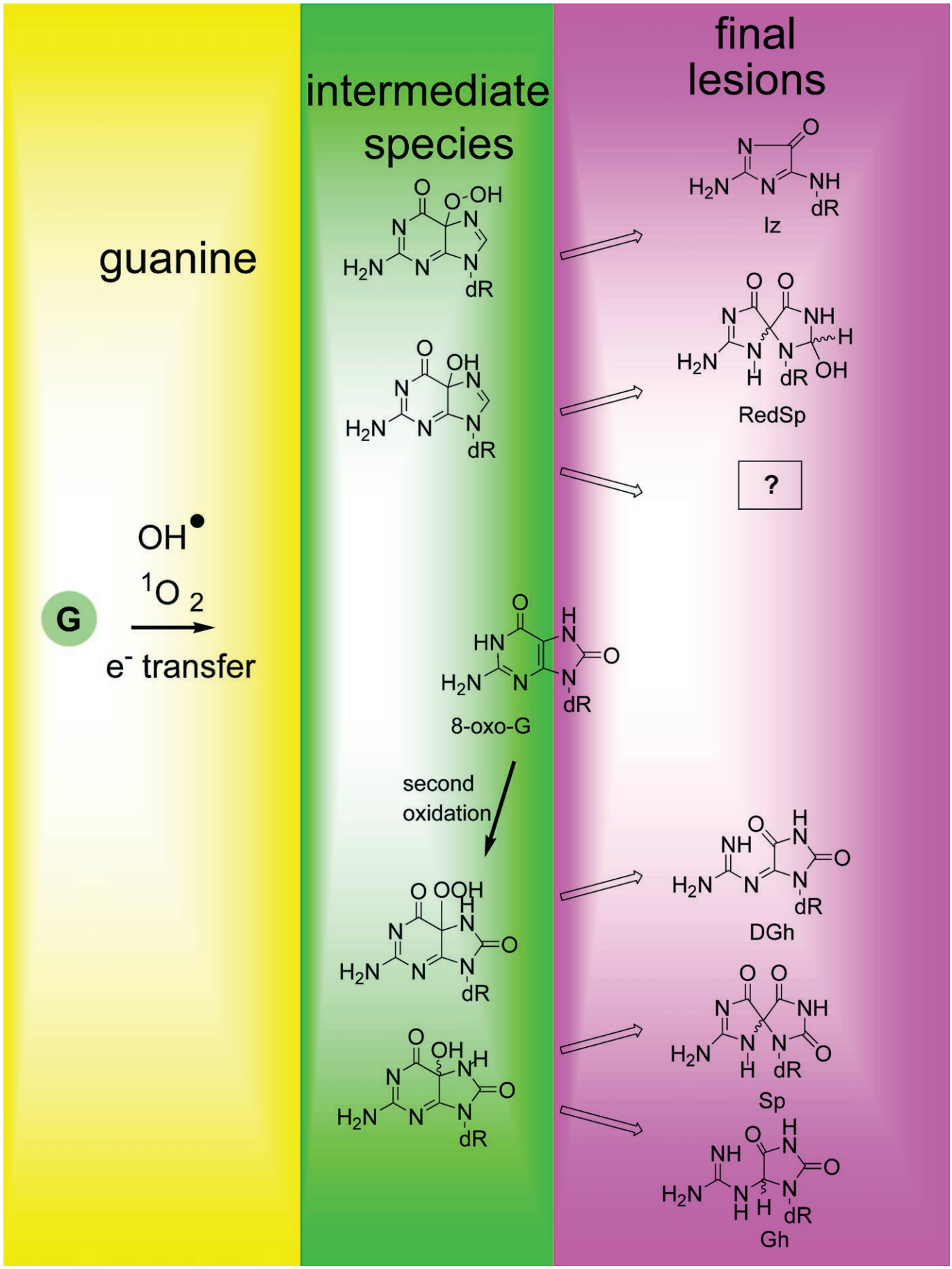

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Guanine Oxidation: One- and Two-Electron Reactions**

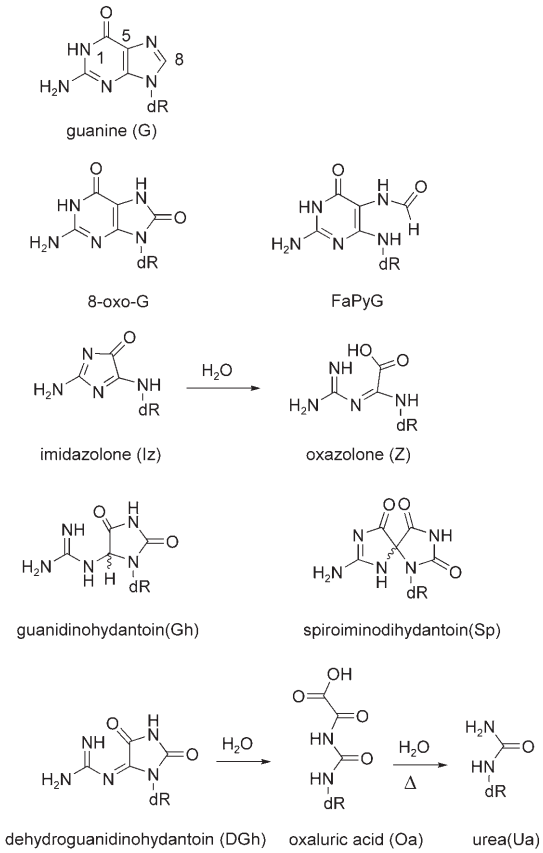
Geneviève Pratviel*[a] and Bernard Meunier*[b]

Abstract: Guanine bases in DNA are the most sensitive to oxidation. A lot of effort has been devoted to the understanding of the chemical modifications of guanine under different oxidizing conditions, the final goal being to know which lesions in DNA can be expected in vivo and their biological consequences. This article analyses the mechanisms underlying guanine oxidation by the comparison between one- and two-electron transfer processes. The different oxidants used in vitro give complementary answers. This overview presents a choice of some key intermediates and the predictive description of G-oxidation products that can be generated from these intermediates depending on the reaction conditions.

Keywords: guanine lesions · guanine oxidation · manganese · nucleosides · porphyrinoids · radical ions

Introduction

Guanine bases are hot spots of DNA damage by oxidation. Guanines are the preferred DNA targets for singlet oxygen and electron-transfer reactions. They are also modified by HO• radicals.[1–4] These different oxidative reactions generate a set of common final lesions (see Scheme 1 for the structures of these chemically modified bases). Initial work on guanine oxidation supported the idea that the formation of 8-oxo-7,8-dihydroguanine (8-oxo-G; see footnote [**] linked to the title of this article) was the main oxidation product, but this evolved to the present situation in which it is only one product among several. On DNA models (nucleosides or short oligonucleotides), imidazolone (Iz; Scheme 1), together with its hydrolysis product, oxazolone (Z), is the main lesion generated by type I photosensitizers (one-electron oxidants)[5–9] and by HO• radicals[5] in the presence of O_2. On the other hand, singlet oxygen favors the formation of a spiroiminodihydantoin derivative, Sp.[10,11] Furthermore,



Scheme 1. The structure of the main guanine oxidation products.

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[**] In the following text, guanine (G) or any oxidized base derived from it will always refer to a residue included in a nucleoside or an oligonucleotide structure. In addition, we will not follow the rules of chemical nomenclature for the numbering of the carbon atoms of the guanine oxidation products but we will keep the numbering of the carbon atoms of the initial G for convenience.

the far easier oxidation of 8-oxo-G (compared to guanine itself) by electron transfer and singlet oxygen allowed the discovery of new lesions (dehydroguanidinohydantoin (DGh), guanidinohydantoin (Gh), spiroiminodihydantoin (Sp)),[12–15] that are also relevant to guanine oxidation.[16–19]

Moreover, the reaction of guanine with a unique strong oxidant (Mn–TMPyP/$KHSO_5$) that is able to mediate a two-electron abstraction reaction proved to be a convenient tool to generate not only all the previously mentioned guanine oxidation products, but also drew attention to new lesions that were not described before.[20–26]

Overall, the parallel study of guanine oxidation by various routes allows a better understanding of the mechanisms of formation of these DNA lesions. Due to common transient intermediates, the different reaction pathways can lead to the same products depending on the reaction conditions. This article analyses the mechanisms underlying guanine oxidation by the comparison between one- and two-electron transfer processes. This overview presents a choice of some key intermediates and the predictive description of G oxidation products that can be generated from these intermediates depending on the reaction conditions.

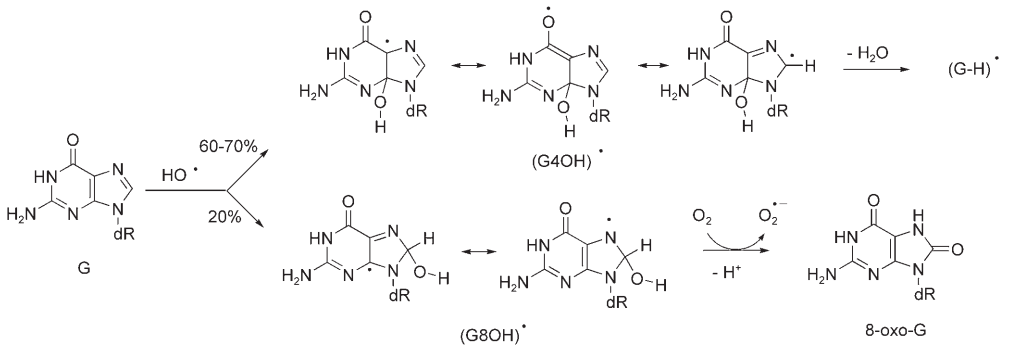
Guanine Oxidation by HO• Radicals

HO• radicals do not react by outer-sphere electron transfer, but by direct addition onto the double bonds of the guanine heterocycle.[27] The action of HO• on G at the level of the nucleoside leads to two intermediate radicals, the hydroxylated radical at C8 (G8OH)• and the neutral guanine radical (G–H)• in ≈20 and ≈70% yield, respectively, relative to HO•.[28] The major product of the reaction of HO• with G, namely (G–H)•, is proposed to be due to the addition of HO• at C4 of G. The (G4OH)• radical is unstable and loses a molecule of H_2O leading to the neutral guanine radical (Scheme 2).[28] The (G–H)• radical has oxidative properties. In contrast, the attack of HO• at C8 of G yields a reducing neutral radical (G8OH)• that reacts quickly with O_2 by electron transfer ($k = 4 \times 10^9\,M^{-1}s^{-1}$) to give rise to 8-oxo-G (Scheme 2).[28] Radicals may be oxidized or reduced depending on the reaction conditions. Thus, alternatively, the (G8OH)• radical may be stabilized by reduction. This reaction pathway leads to the formamidopyrimydine derivative of guanine (FaPyG) (Scheme 1).[1–4,29] Similarly, the oxidation of the (G–H)• will be considered later. Within double-stranded DNA exposed to the HO• radical, the main decomposition product of G was found to be 8-oxo-G.[1,2]

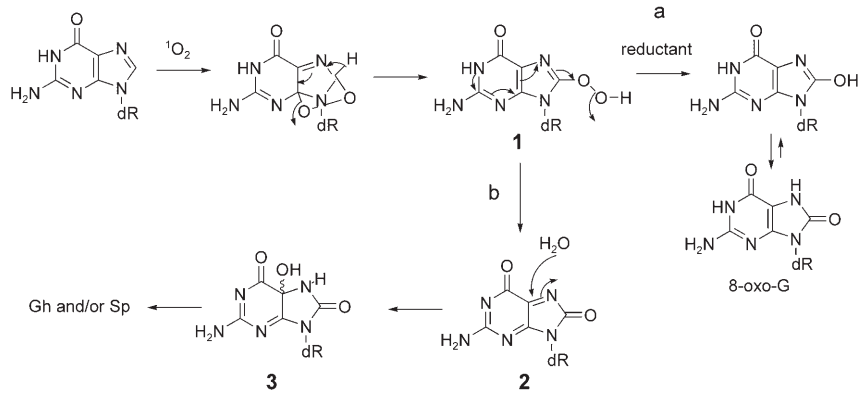
The neutral guanine radical (G–H)• is the conjugated base of the guanine radical cation G•+ ($pK_a = 3.9$).[27] Thus, it appears that (G–H)•, which is the first intermediate of guanine oxidation by one-electron transfer, is also one intermediate of guanine oxidation by HO• radicals. In summary, guanine oxidation by HO• radicals will converge to the same products as for the oxidation by electron transfer or by singlet oxygen through the formation of (G–H)• and 8-oxo-G (see below for details).

Oxidation of G and 8-Oxo-G by Singlet Oxygen

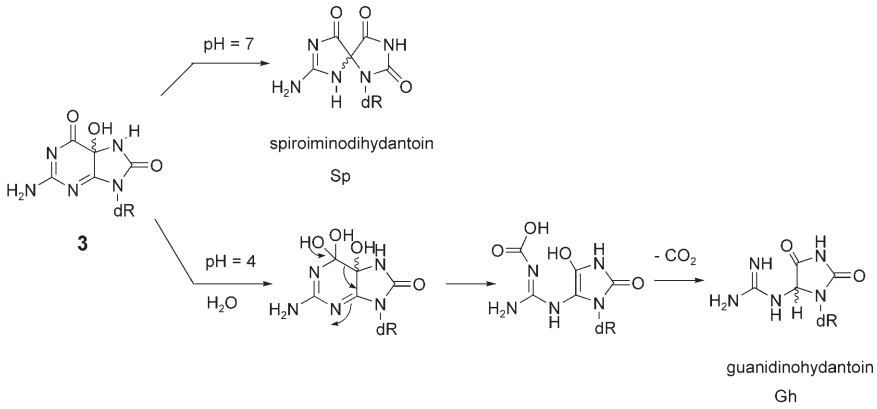
In the presence of singlet oxygen, guanine base undergoes a [4+2] cycloaddition as shown on Scheme 3. The endoperoxide rearranges into a 8-hydroperoxy derivative (**1**), which is an oxidant as strong as a peracid and is expected to be reduced into a 8-hydroxylated residue, the tautomeric form of 8-oxo-G (Scheme 3, pathway a).[30] This reaction pathway is in agreement with the fact that 8-oxo-G was found in cellular DNA after treatment with $^1O_2$.[31] The oxygen atom incorporated in 8-oxo-G originated from labeled singlet oxygen $^1(^{18}O)_2$.[31] Alternatively, in the absence of a reducing agent, **1** has been recently shown to lead to an intermediate oxidized species of 8-oxo-G (**2**) (Scheme 3, pathway b) that is prone to the attack by nucleophiles (the most abundant one being H_2O under usual reaction conditions) at C5 to give the intermediate **3**. The transient species **3** is the common precursor of Sp and Gh.[32] The ratio between Sp and Gh depends on the pH of the reaction (Scheme 4).[14,15] The formation of Gh is favored at acidic pH (pH 4), whereas the Sp prevails at higher pH (pH 7). The rearrangement of **3** into Sp is not favored in double-stranded DNA. On the other hand, oxidation of 8-oxo-G by $^1O_2$ yields DGh after a [2+2] cycloaddition and the formation of intermediate **4** as shown in Scheme 5.[13] A different rearrangement of the en-



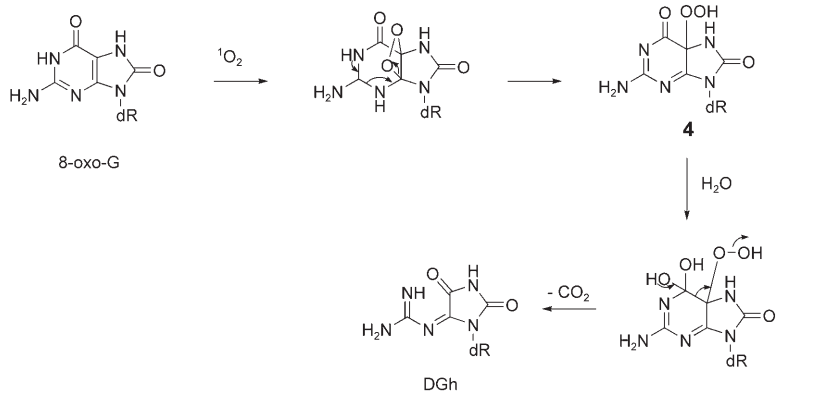
Scheme 2. The mechanism of guanine oxidation by HO• radicals. Independent formation of 8-oxo-G and the neutral guanine radical. dR stands for a deoxyribose unit.



Scheme 3. Guanine oxidation by singlet oxygen. Products are formed from a hydroperoxide intermediate at C8 (**1**).



Scheme 4. Formation of Sp and Gh from **3**.



Scheme 5. Oxidation of 8-oxo-G by singlet oxygen. Formation of DGh from a peroxide intermediate at C5 (**4**).

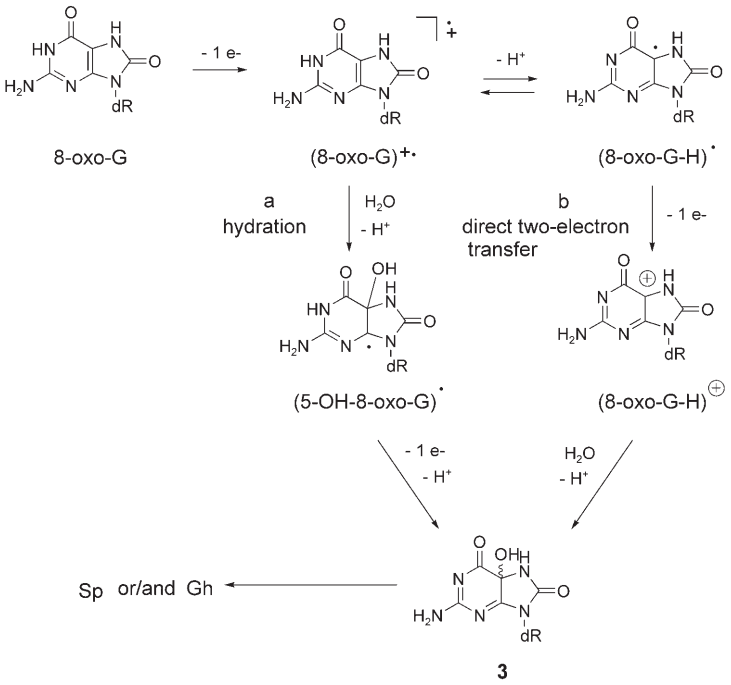
doperoxide was proposed in organic solvent[30] and may not be relevant to aqueous media. Cyanuric acid derivative was also reported to form in the reaction of 8-oxo-dG with $^1O_2$,[33] but the mechanism of its formation in aqueous solution awaits clarification.

The four products described in this paragraph, namely 8-oxo-G, Gh, Sp, and DGh, are typical products of guanine oxidation by electron transfer. Intermediate species **1**, **3**, and **4** are also found in different mechanistic schemes related to electron transfer.

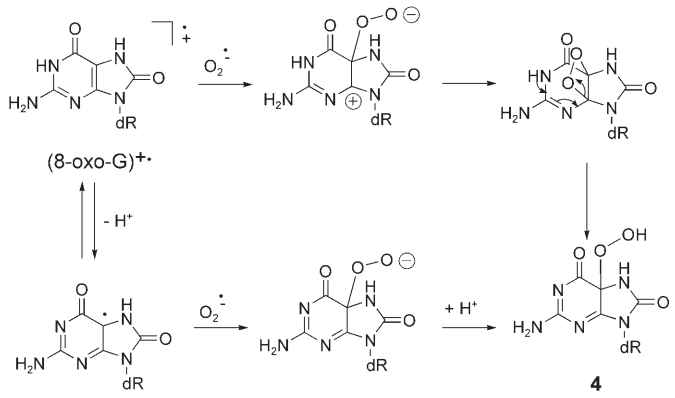
Oxidation of 8-oxo-G and G by Electron Transfer

Oxidation of 8-oxo-G by electron transfer: The modified base 8-oxo-G is more easily oxidized than G in any DNA-sequence context. The redox potential of 8-oxo-G (0.74 V versus NHE)[34] is lower than that of G (1.29 V versus NHE).[35] Thus 8-oxo-G is preferentially oxidized when competition with G is considered.[30,36] After the abstraction of one electron, the intermediate 8-oxo-G radical-cation, $(8\text{-oxo-G})^{\bullet+}$, is prone to deprotonation to give the neutral form $(8\text{-oxo-G}{-}H)^{\bullet}$ ($pK_a = 6.6$).[34] This neutral radical can be observed by transient absorption spectroscopy.[37]

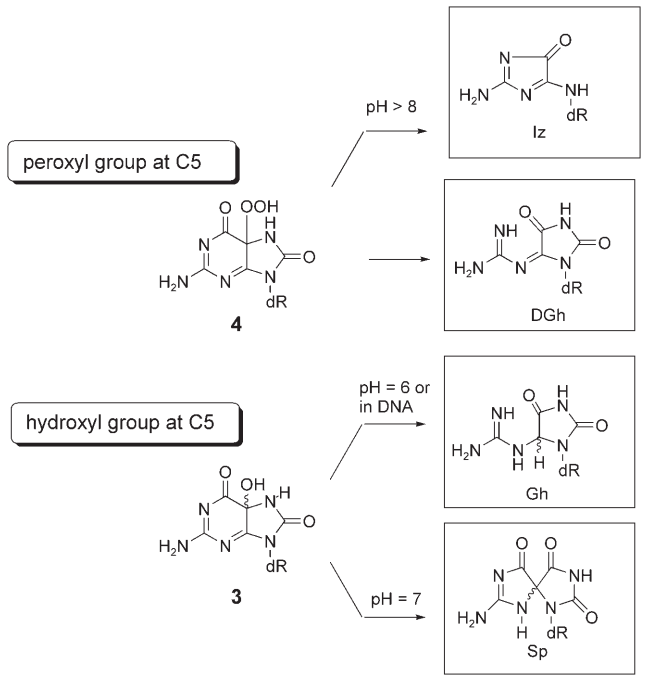
With an oxidant such as Na_2IrCl_6 (redox potential of $Ir^{IV}Cl_6^{2-}/Ir^{III}Cl_6^{3-}$ at neutral pH is 0.86 V versus NHE) the oxidation of 8-oxo-G proceeds through a two-electron oxidation process (Scheme 6).[14,15] The oxidation may proceed by two sequences of events that could not be distinguished (pathways a and b in Scheme 6). The attack of a water molecule is possible on $(8\text{-oxo-G})^{\bullet+}$ (Scheme 6, pathway a) and this pathway is also referred to as hydration of the radical cation. This route produces, after deprotonation of the adduct, a neutral radical species $(5\text{-OH-8-oxo-G})^{\bullet}$, which can be further oxidized (second electron abstraction) to generate **3**. In the same manner as described in Scheme 2, in which the $(G8OH)^{\bullet}$ radical is more oxidizable than $(G{-}H)^{\bullet}$, the hydroxylated neutral radical, $(5\text{-OH-8-oxo-G})^{\bullet}$, may be easier to oxidize than $(8\text{-oxo-G}{-}H)^{\bullet}$ and may undergo another oxidation step by electron transfer to O_2. Intermediate **3** collapses into Gh or/and Sp as shown in Scheme 4.[14,15,32] Alterna-



Scheme 6. Oxidation of 8-oxo-G by a two-electron transfer mechanism. Products are formed from a hydroxylated intermediate at C5 (**3**).



Scheme 7. One-electron oxidation of 8-oxo-G. Radical–radical combination and trapping of the radical cation or the neutral radical by superoxide anion.



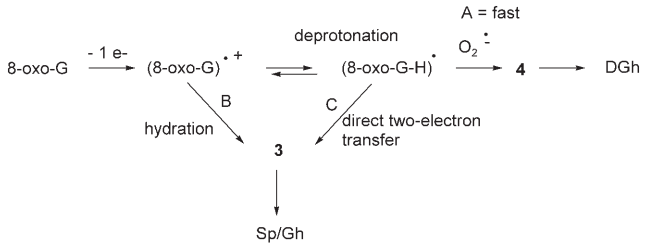
Scheme 8. The products of 8-oxo-G oxidation by electron transfer formed from the two key intermediates **3** and **4**.

tively (Scheme 6, pathway b), a second electron can be abstracted from (8-oxo-G–H)• by an oxidant. This would lead to an short-life cationic entity $(8\text{-oxo-G–H})^+$ in which the positive charge is located at C5. That cation can then be trapped by a nucleophilic species, in particular H_2O. Such attack at C5 by a molecule of water would lead to intermediate **3**. Pathway b can be referred to as a direct two-electron oxidation, since the oxidant abstracts two electrons from G in two steps. Labeling experiments confirm the attack of a water molecule at C5 by the incorporation of an oxygen atom from a molecule of $H_2{}^{18}O$ in the reaction products with Na_2IrCl_6 (Sp and Gh).[14,15]

The quenching of the radical due to a simple one-electron abstraction, (8-oxo-G–H)• (or 8-oxo-G$^{\bullet+}$), by another radical species, namely $O_2^{\bullet-}$, is unambiguously observed during the oxidation of 8-oxo-G by the triplet excited state of riboflavin (a type I photosensitizer, considered as a one-electron oxidant).[38] The production of $O_2^{\bullet-}$ in the reaction medium is due to the regeneration of riboflavin by electron transfer onto O_2 (the electron abstracted from G residues by the excited state of riboflavin creates a radical anion form of the photosensitizer). This radical–radical combination (i.e. reaction between two different radicals) is interesting, because it can induce the formation of different products with respect to the two-electron transfer described in Scheme 6. The quenching of (8-oxo-G–H)• (or (8-oxo-G)$^{\bullet+}$) by $O_2^{\bullet-}$ is shown in Scheme 7. Intermediate **4** collapses into DGh by a pathway reminiscent of the $^1O_2$ oxidation of 8-oxo-G (Scheme 5). As summarized in Scheme 8 the hydroperoxide intermediate at C5 (**4**) leads to DGh (Scheme 5) and the hydroxylated intermediate at C5 (**3**) leads to Sp/Gh (Scheme 4). It should be noted, at pH values above 8, intermediate **4** is prone to transformation into Iz. The mechanism probably involves an intramolecular nucleophilic attack of the peroxide group.[38] In contrast to the two-electron oxidation process, the formation of **4** illustrated in Scheme 7 (trapping of the radical intermediate by $O_2^{\bullet-}$) corresponds to the abstraction of only one electron from the initial 8-oxo-G by the oxidant. Addition of superoxide dismutase (SOD) induces an increase of Sp/Gh (produced from **3**) and a concomitant decrease of DGh (or Iz at pH 8.6) (formed from **4**).[38] The reaction proceeds in the same way on double-stranded oligonucleotides at pH 7 or pH 7.5, at which DGh was found as the major product of 8-oxo-G oxi-

dation by type I photosensitizers[9,39] and contained an oxygen atom from O_2.[9]

These data can be rationalized by a competition between the one-electron oxidation pathway (Scheme 9, A) and the two-electron oxidation pathway (Scheme 9, B and/or C).



Scheme 9. Competition between hydration and deprotonation of the radical-cation of 8-oxo-G.

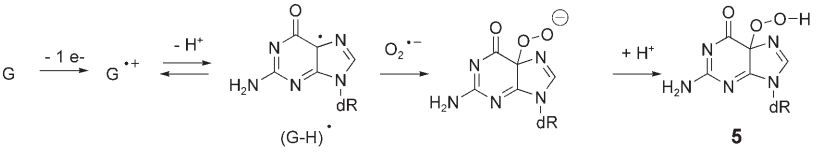
Deprotonation, that is, a one-electron oxidation mechanism, would lead to the trapping of intermediate radical species by $O_2^{\bullet-}$ (formation of intermediate **4** and production of DGh). The two-electron oxidation mechanism (hydration or direct two-electron oxidation) would result in the trapping of cationic species by nucleophiles, in this case H_2O, at C5 (formation of intermediate **3** and production of Sp/Gh). Pathway A in Scheme 9 is of course not observed in the case of the oxidation with Na_2IrCl_6, since the reaction medium does not contain any radical trap (Scheme 6).[14,15] In that case only products derived from two-electron oxidation mechanism (i.e., from **3**) are observed. On the other hand, since the trapping by $O_2^{\bullet-}$ is very efficient (see below for details), type I photosensitizers generally react by a one-electron oxidation process, leading only to the formation of products issued from **4**.

The combination of $O_2^{\bullet-}$ with the (8-oxo-G–H)$^\bullet$ in double-stranded DNA and the fate of the neutral radical (8-oxo-G–H)$^\bullet$ in the absence of $O_2^{\bullet-}$ has been studied on oligonucleotides at pH 7.5.[40] The reaction between (8-oxo-G–H)$^\bullet$ and $O_2^{\bullet-}$ is fast, $k = 1.0\times10^8\,\text{M}^{-1}\text{s}^{-1}$, in double-stranded DNA as well as in single-stranded DNA, $k = 1.3\times10^8\,\text{M}^{-1}\text{s}^{-1}$. It should be noted that (8-oxo-G–H)$^\bullet$ does not react quickly with O_2 ($k < 10^2\,\text{M}^{-1}\text{s}^{-1}$). The neutral radical has been generated by photoexcitation of a 2-aminopurine (2-AP) residue incorporated in an oligonucleotide. A two-photon ionization of 2-AP gives rise to a 2-AP radical that is able to selectively oxidize 8-oxo-G within the oligonucleotide. The hydrated electrons derived from photoionization of 2-AP are quantitatively scavenged by O_2 to generate superoxide radical anion. When superoxide dismutase (SOD) is added the yield of DGh becomes negligible and the (8-oxo-G–H)$^\bullet$ radical decays on the time interval of several seconds. The major reaction product is then Sp. The proposed mechanism involves the reaction of a water molecule with the neutral radical (8-oxo-G–H)$^\bullet$, in the presence of SOD, but the reaction of a nucleophile with a neutral radical is not possible. Since the spiro derivative is produced from the hydroxylated intermediate **3**, this result may be in accordance with a two-electron oxidation of 8-oxo-G, occurring only in the presence of SOD.

In summary, two key intermediates emerge in the formation of 8-oxo-G oxidation products: **3** and **4**. Different oxidation products result from each of them (Scheme 8). The partitioning between these two pathways (leading to either **3** or **4**) depends on the capacity of the oxidant to perform a two- or a one-electron oxidation, respectively (Scheme 9). The two-electron oxidation of 8-oxo-G by Na_2IrCl_6 instead of one-electron oxidation by type I photosensitizers is interesting and useful, because it provides the opportunity to observe and characterize the oxidation products arising from **3**. Under some particular reaction conditions, especially in vivo, the products arising from **3** might form. One may envision for instance the reduction of the hydroperoxide (**4**) into the hydroxylated derivative (**3**),[41] or one cannot exclude the radical–radical combination between the radical due to a one-electron abstraction, (8-oxo-G–H)$^\bullet$ (or 8-oxo-G$^{\bullet+}$) and HO$^\bullet$ giving directly **3**. One- and two-electron reactions complement each other. This will be illustrated also in the case of guanine.

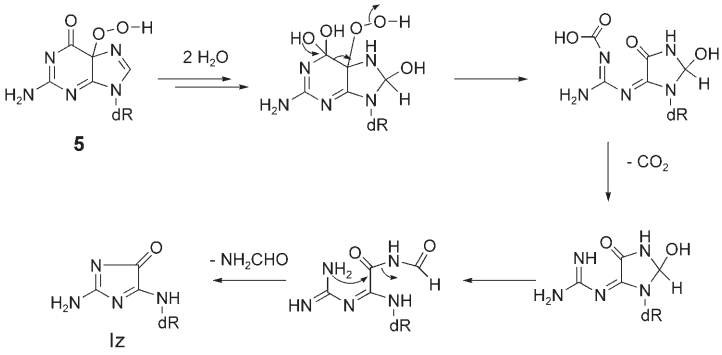
Oxidation of G by electron transfer: The abstraction of one electron from G generates the guanine radical cation G$^{\bullet+}$. After its fast deprotonation,[42] this radical can be observed in DNA.[43–46] It reacts with other radical species (radical–radical combination) present in the reaction medium; O_2 was proposed due to its partial radical character,[5] but the reaction is slow ($k < 10^6\,\text{M}^{-1}\text{s}^{-1}$). A kinetically more favored mechanism is the quenching of this guanine neutral radical (G–H)$^\bullet$ by superoxide radical anion, $O_2^{\bullet-}$ (estimated $k = 3\times10^9\,\text{M}^{-1}\text{s}^{-1}$ for nucleosides).[29] The quenching of (G–H)$^\bullet$ by $O_2^{\bullet-}$ occurs with a rate constant $k = 4.7\times10^8\,\text{M}^{-1}\text{s}^{-1}$ in double-stranded oligonucleotides.[39] The radical–radical combination of (G–H)$^\bullet$ with radicals is generally fast. For example, the reaction of $NO_2^\bullet$ with (G–H)$^\bullet$ in double-stranded DNA takes place with a rate constant of $k \approx 4.3\times10^8\,\text{M}^{-1}\text{s}^{-1}$.[47] The reactivity of (G–H)$^\bullet$ with $Me_3C^\bullet$ was also reported to be high, close to diffusion rate ($k \approx 10^9\,\text{M}^{-1}\text{s}^{-1}$).[28]

The quenching of (G–H)$^\bullet$ by $O_2^{\bullet-}$ is followed by protonation of the adduct and gives rise to a hydroperoxide adduct at C5 (**5**; Scheme 10). The oxidation of 2′-deoxyguanosine by benzophenone (a type I photosensitizer) under aerated



Scheme 10. The bimolecular combination of the neutral guanine radical with $O_2^{\bullet-}$.

conditions allows the characterization of imidazolone (Iz), the major guanine oxidation product derived from **5** (Scheme 11).[5,6] This mechanism has been confirmed by the

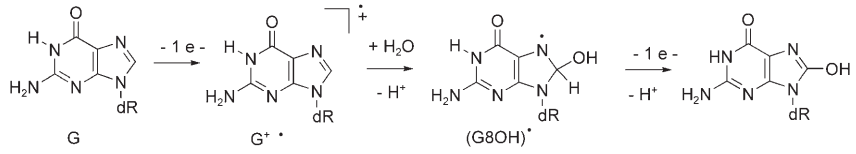


Scheme 11. Formation of Iz from a hydroperoxide intermediate at C5.

incorporation of one O-atom from labeled $^{18}O_2$ in Iz.[5] The mechanism of the formation of Iz can be considered as a one-electron oxidation process, since only one electron is abstracted from G by the oxidant. This mechanism stands true for riboflavin, another type I photosensitizer.[9] It is reminiscent of the one described in Scheme 7 for 8-oxo-G. Intermediate **5** is one of the key intermediates involved in the different mechanisms of guanine oxidation. Iz is also the major product of guanine oxidation in double-stranded oligonucleotides.[8,9,39]

The trapping of the radical cation $G^{\bullet+}$ by H_2O (referred to as hydration of $G^{\bullet+}$)[27] is commonly accepted as being at the origin of the formation of 8-oxo-G. The slower release of the proton on N1, involved in base-pairing, could explain the formation of 8-oxo-G in double-stranded DNA and not in single-stranded DNA. Due to its low pK_a, the proton on N1 of the radical cation is immediately lost in the case of nucleosides and single-stranded DNA. Consequently, it has been proposed that in single-stranded DNA the products of reaction arise from deprotonation and reaction of $(G-H)^{\bullet}$ with $O_2^{\bullet-}$, whereas in double-stranded DNA hydration of $G^{\bullet+}$ is kinetically possible. From DFT calculations a water molecule is able to attack the guanine radical cation $G^{\bullet+}$ at C8, but does not react with the neutral radical $(G-H)^{\bullet}$.[48] Indeed, the neutral radical $(G-H)^{\bullet}$ does not give rise to 8-oxo-G.[28]

Hydration of $G^{\bullet+}$ at C8 produces the neutral radical $(G8OH)^{\bullet}$ (Scheme 12). This neutral radical is the same as that obtained from the reaction of $HO^{\bullet}$ with G (Scheme 2). As described before, the formation of 8-oxo-G requires the



Scheme 12. Formation of 8-oxo-G by a two-electron transfer.

abstraction of a second electron and one proton from $(G8OH)^{\bullet}$. Thus, the formation of 8-oxo-G by electron transfer in double-stranded DNA corresponds to a two-electron oxidation process through the formation of an intermediate reducing radical $(G8OH)^{\bullet}$. The first electron is abstracted by the oxidant and the second one might be simply abstracted by O_2. This mechanism was supported by the incorporation of one labeled oxygen atom from $H_2{}^{18}O$ in 8-oxo-G in double-stranded DNA.[49]

Recently, the deprotonation rate of $G^{\bullet+}$ within double-stranded DNA was measured in deoxygenated solutions.[42] The conversion from $G^{\bullet+}$ to $(G-H)^{\bullet}$ in double-stranded DNA takes place on timescale similar to that observed with 2′-deoxyguanosine. The radical cation of 2′-deoxyguanoine deprotonates into the neutral guanine radical with a rate constant of $1.8\times10^7\,s^{-1}$. The deprotonation of the guanine radical cation in double-stranded DNA has been described as a two-phase process with rate constants of $\approx1.3\times10^7\,s^{-1}$ and $\approx3\times10^6\,s^{-1}$ for the faster and slower phases, respectively. The two phases have been attributed to the deprotonation of the $G^{\bullet+}$ moiety in the G:C base pair in the form of $G^{\bullet+}$:C for the first and fast phase and to the deprotonation of the $(C+H)^+$ into $(G-H)^{\bullet}$:$(C+H)^+$ for the second and slower phase. Consequently, the differences in the product distribution observed in single- versus double-stranded DNA may not be attributed only to a longer life span of $G^{\bullet+}$ in the double helix. An alternative proposal would be to consider that the local concentration of the negatively charged $O_2^{\bullet-}$ in the vicinity of double-stranded DNA would be lower, or that the guanine radical would be less accessible within double-stranded DNA (particularly the C5 position). In analogy with the discussion related to Scheme 9, the fast radical–radical combination between $(G-H)^{\bullet}$ (or $G^{\bullet+}$) with $O_2^{\bullet-}$ would drive the protonation/deprotonation equilibrium of $G^{\bullet+}/(G-H)^{\bullet}$ to the right so that all the guanine oxidation products would arise from **5**. On the other hand, if the concentration of $O_2^{\bullet-}$ decreases the rate of the radical–radical reaction would slow down, whereas the rate of hydration would not, since this reaction can be considered as a reaction of the first order in water. This would explain why the ratio between Iz and 8-oxo-G was different depending on the experimental conditions. On calf thymus DNA or on double-stranded oligonucleotides modified by riboflavin oxidation, Iz appears as the major guanine oxidation product whereas 8-oxo-G accounts for a minor product calculated on consumed G.[8,9,39] On the opposite, at a low level of DNA damage (low $^1O_2$ concentration), the ratio between Iz and 8-oxo-G has been reported as being around 1.[2,50]

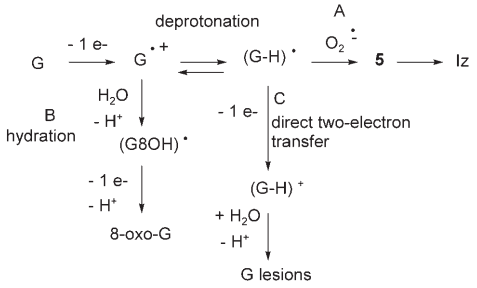
The formation of Iz as a major product implies that the quenching of the guanine radical by $O_2^{\bullet-}$ mainly occurs at C5 of guanine. However, in the case of the trapping of the intermediate guanine radical by $NO_2^{\bullet}$ in a double-stranded oli-

gonucleotide, trapping at C5 and C8 account for 70 and 30%, respectively.[47] The trapping of guanine radical by $O_2^{\bullet-}$ at C8 would lead to intermediate **1** (Scheme 3). It should be noted that the hydration mechanism has only been revealed by the reaction of a water molecule at C8 of $G^{\bullet+}$ and not at C5.

As in the case of 8-oxo-G oxidation, the extremely efficient trapping of the intermediate guanine radical by $O_2^{\bullet-}$ may preclude the observation of a two-electron oxidation mechanism. Since most of the used oxidants $SO_4^{\bullet-}$,[42] $Br_2^{\bullet-}$,[51] $CO_3^{\bullet-}$,[18] and type I photosensitizers[5,7,8,49,52–55] are intrinsic radicals or generate radical species in the reaction medium, the intermediate radical of guanine reacts preferentially by a fast bimolecular combination of radicals. The addition of SOD in the case of a type I photosensitizer dramatically enhances the lifetime of guanine radicals from 4–7 ms to 0.2–0.6 s in double-stranded oligonucleotides.[39] This means that the hydration of $G^{\bullet+}$ (Scheme 12) is much slower than the radical–radical combination.

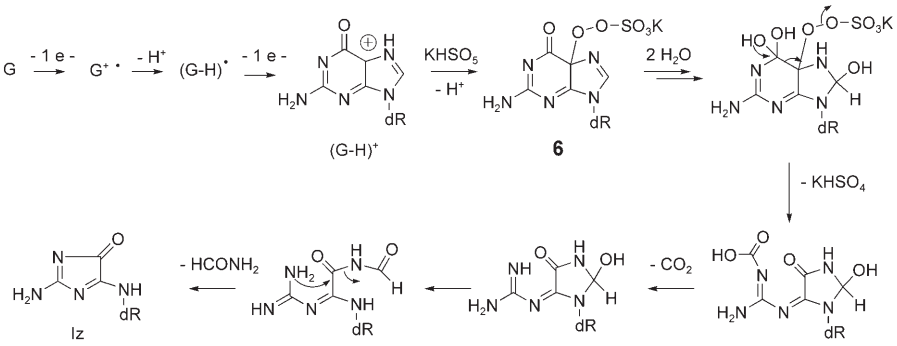
The oxidation of G without the intervention of any radical trap has been performed with the strong chemical oxidant based on the association of a manganese–porphyrin, bis-(aqua)-*meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrinato-manganese(III) (Mn–TMPyP) with $KHSO_5$ as an oxygen atom donor, Mn–TMPyP/$KHSO_5$ for short.[20–26] The oxidative species, a high-valent Mn^V=O entity, is able to perform the two-electron oxidation of G. The two-electron transfer mechanism for guanine oxidation by Mn–TMPyP/$KHSO_5$ has been initially evidenced with 2′-deoxyguanosine.[20] Incubation of dG with Mn–TMPyP/$KHSO_5$ produces Iz in nearly quantitative yield (90%). However, in contrast to what has been reported for the mechanism of Iz formation by one-electron transfer (Scheme 11), the formation of Iz with Mn–TMPyP/$KHSO_5$ is not dependent upon the presence of molecular oxygen. When the reaction is performed in the presence of labeled molecular oxygen ($^{18}O_2$), Iz is not labeled. The fact that no intermediate radical species of guanine has been trapped by any radical (no free radicals are present in the reaction medium) allows this intermediate radical of guanine to undergo a second one-electron oxidation. With such a strong oxidant the two one-electron steps cannot be distinguished, making this metal–oxo species a very efficient two-electron transfer system. Abstraction of one electron from $(G-H)^{\bullet}$ produces a nonradical cationic species, $(G-H)^+$. By analogy with the one- and two-electron reactions summarized in Scheme 9 for 8-oxo-G, Scheme 13 illustrates one- and two-electron reactions for G.

The guanine radical reacts with other radical species, while the guanine cation $(G-H)^+$ is highly electrophilic and



Scheme 13. Competition between different pathways of guanine oxidation by electron transfer. One-electron transfer, pathway A, and two-electron transfer, pathways B (hydration) and C (direct two-electron transfer). G lesions may include 8-oxo-G and RedSp or RedGh.

reacts with nucleophilic molecules. However, when the reaction is performed in labeled water ($H_2{}^{18}O$), Iz does not incorporate a labeled ^{18}O atom from the water. Consequently, the only rational origin for the oxygen atom at the C5 position of G is the peroxide itself, $KHSO_5$. Peroxides are better nucleophiles than water molecules. These labeling studies unambiguously indicate that, with Mn–TMPyP/$KHSO_5$, the oxidation of guanine is a direct two-electron oxidation. The mechanism of the formation of Iz by means of the two-electron oxidation of G is shown in Scheme 14. The intermedi-

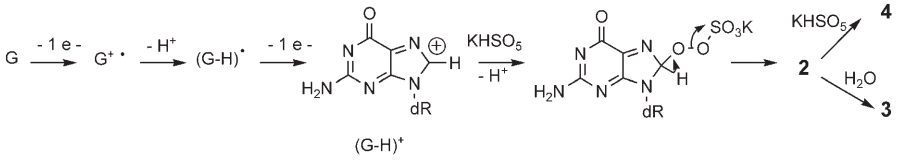


Scheme 14. Formation of Iz by the trapping of the $(G-H)^+$ intermediate by $KHSO_5$ at C5.

ate nonradical cationic derivative of guanine, $(G-H)^+$, is trapped by the most electrophilic entity in the reaction medium, namely $KHSO_5$. Several steps from **6**, identical to the ones described in Scheme 11 from **5**, produce the imidazolone derivative after the release of formamide, which has also been detected.[20] It is worth noting that the peroxide substituent contains, in the case of **6**, a good leaving group (KSO_4^-) making easy the heterolytic cleavage of the O–O bond at C5.

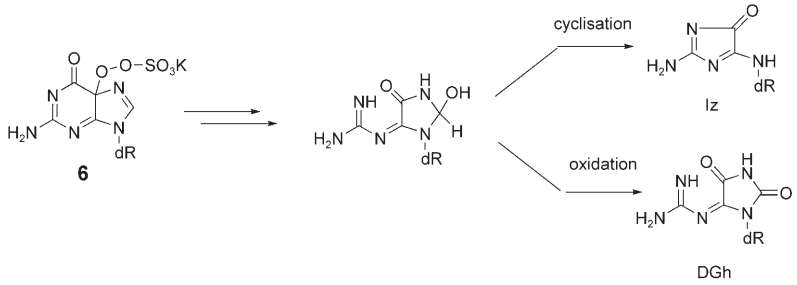
These data point to the fact that the C5 position of the $(G-H)^+$ species is the most reactive as also observed in the case of $(G-H)^{\bullet}$.[5] This behavior is probably due to the stability of the reaction products (that are the same). However, a minor reaction of $KHSO_5$ at the C8 of $(G-H)^+$ would have yielded an oxidized 8-oxo-G derivative (**2** in Scheme 3). The observed small amount (<10%) of DGh may be formed by

such reaction pathway under the used experimental conditions (high $KHSO_5$ concentration). The mechanism probably involves the attack of $KHSO_5$ at C5 of the electrophilic intermediate **2** leading to **4** (Scheme 15).



Scheme 15. Trapping of the $(G-H)^+$ intermediate by $KHSO_5$ at C8 (minor mechanism).

On double-stranded oligonucleotides the major product of guanine oxidation by Mn-TMPyP/$KHSO_5$ is usually DGh. During a labeling experiment with a reaction mixture containing $H_2{}^{18}O$, this oxidation product has been identified as a mixture of three labeled species containing either, two ^{18}O, two ^{16}O, or one ^{18}O + one ^{16}O atoms. Consequently, the mechanism of its formation is plurimodal and will not be detailed here (see references [23, 25] for details). Briefly, oxidation at C8 by Mn^{V}=O operated by oxygen-atom transfer prior to a cyclization into Iz can produce DGh in double-stranded DNA (Scheme 16). The structure of DGh has been



Scheme 16. Formation of DGh instead of Iz by C8 oxidation. DGh is not a precursor of Iz.

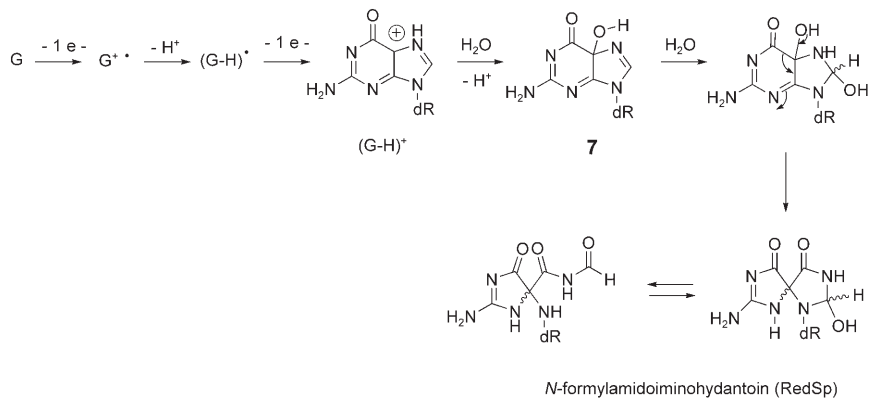
confirmed by the NMR identification of its hydrolysis product Oa (or urea under more drastic conditions) and its reduction product Gh (See Scheme 1 for structures).[23–25]

A minor amount of Gh and Sp has also been observed. The labeling of these two derivatives is in accordance with the incorporation of at least one ^{18}O atom from $H_2{}^{18}O$ in their structure, logically at C5, since these two products arise from intermediate **3**. Also for these two compounds, the labeling at C8 is a mixture suggesting different oxidation reactions.

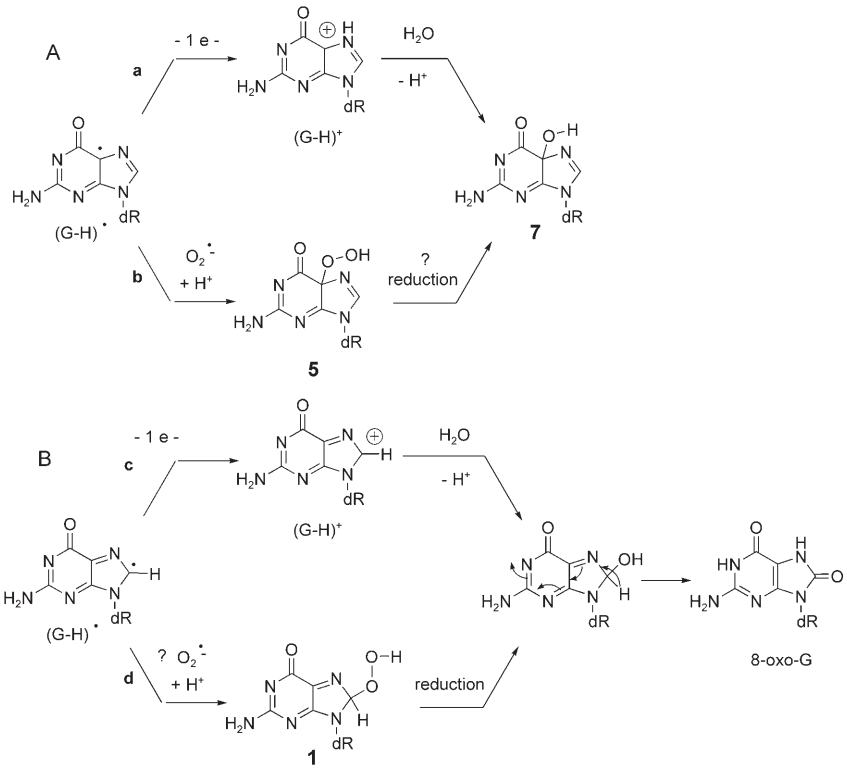
A more interesting product, although in a minor amount, is a product of guanine oxidation showing an increase in mass of 34 mass units compared to the mass of G.[21,22] Such mass increase results from the incorporation of two ^{18}O atoms from $H_2{}^{18}O$.[21,22] One of these ^{18}O atoms is exchangeable with H_2O during chromatography. From what we know on the general mechanisms of guanine oxidation, this compound must arise from the hydroxylation at C5 of the $(G-H)^+$ intermediate, leading to an unstable compound **7** with one labeled oxygen atom from $H_2{}^{18}O$ at C5 (Scheme 17). This intermediate is expected to rearrange at physiological pH into a spiro structure like **3** in Scheme 4. We propose the addition of a water molecule at C8 (Scheme 17) in a manner similar to the opening of the five-membered ring in the formation of Iz (Scheme 11 and Scheme 14). An equilibrium between a closed and open form provides an explanation for the exchange of label with solvent during chromatography. This compound could be referred to as an *N*-formylamidoiminohydantoin derivative or RedSp to take into account the non-oxidized state of C8. The full characterization of RedSp awaits NMR data. A guanine oxidation product with the same molecular mass has been previously reported in guanine oxidation with a binuclear copper complex.[56] Nevertheless, this compound has not been observed before with traditional one-electron oxidants, because it arises from a hydroxylated intermediate at C8 (**7**) that has not been observed in the hydration mechanism.

As we proposed earlier in the case of 8-oxo-G, hydroxylated precursors of guanine oxidation products (such as **7**) can be observed in the case of a two-electron oxidation of G in the absence of superoxide anion or other efficient radical trap (Scheme 18 A, pathway a). However, in the case of one-electron oxidation of G, the reduction of the peroxide intermediate at C5 (**5**) into **7** may be proposed (Scheme 18 A, pathway b), but this may not be competitive with the formation of Iz, which is rapid. On the other hand, if H_2O trapping occurs at C8 the two-electron mechanism leads to 8-oxo-G (Scheme 18 B, pathway c). Alternatively, the reduction of an intermediate hydroperoxide **1** is also possible (Scheme 18 B, pathway d). It has been proposed to be at the origin of the formation of 8-oxo-G with $^1O_2$ (Scheme 3). The reaction of the radical intermediate with $HO^{\bullet}$ is not represented in Scheme 18, but might be considered for **7** being a product of one-electron oxidation of G. Thus the two-electron oxidation of G with Mn–TMPyP/$KHSO_5$ affords suitable conditions to observe hydroxylated intermediates during G oxidation. In the same way, hydroxylated intermediates during 8-oxo-G oxidation are observed with Na_2IrCl_6 which is able to mediate a two-electron oxidation of 8-oxo-G.

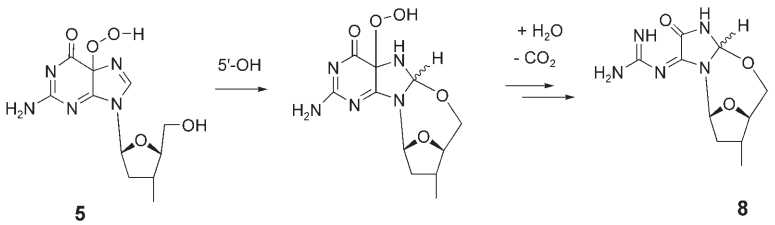
In contrast to what is observed in the oxidation of 8-oxo-G, the products of G oxidation are not necessarily oxidized at the C8 position, since they arise from **5** in Scheme 11 or **7** in Scheme 17. However, when an electron-withdrawing sub-



Scheme 17. Proposed mechanism for the formation of *N*-formylamidoiminohydantoin (RedSp) by the trapping of the $(G-H)^+$ intermediate by H_2O.



Scheme 18. A) Guanine oxidation by one- or two-electron oxidation reaction when the trapping of the radical occurs at C5. B) Proposed guanine oxidation by one- or two-electron oxidation reaction when the trapping of the radical occurs at C8. This mechanism is less favored than that shown in A.



Scheme 19. Attack of a 5′-OH group at the C8 of **5**. The base is in an *anti* conformation. The O atom of the sugar is above the plane.

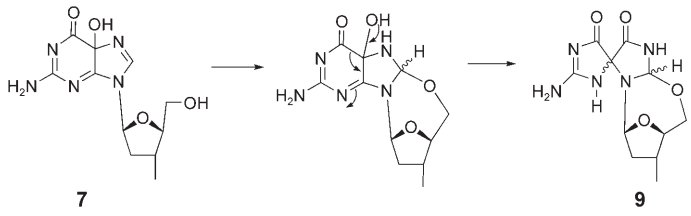
stituent is bound at C5, the C8 position of G becomes electrophilic. This is illustrated by the attack of a molecule of water at C8 in the mechanism of Iz formation (Scheme 11 and Scheme 14). Similarly, a cyclic compound generated by attack of a 5′-OH group instead of a molecule of water at C8 of **5** has also been observed[36] (**8** in Scheme 19) as well as a similar one resulting of the attack of a 5′-amino group.[57,58]

The attack of a molecule of water at C8 of **7** is illustrated in Scheme 17. Another product arising from a second nucleophilic attack at C8 is due to the attack of the neighboring 5′-OH group from the 5′-end of an oligonucleotide (**9** in Scheme 20).[26] This pathway is favored by an *anti* conformation of the base in the double helix of DNA.

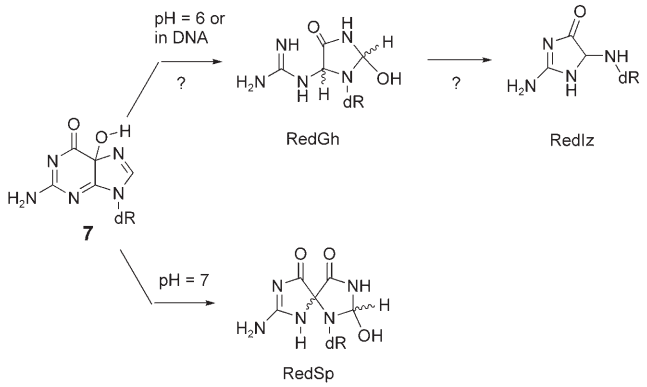
Intermediate **7** for guanine oxidation corresponds to intermediate **3** for the 8-oxo-G (Scheme 6). As **3** gives rise to Gh and Sp, **7** should give rise to RedSp and RedGh, depending on the pH of the reaction mixture (Scheme 21). Under the reaction conditions with Mn–TMPyP/$KHSO_5$ ($pH \geq 7$ and strong oxidant) neither RedGh nor the related putative RedIz were detected. However, RedGh is likely to be formed under appropriate reaction conditions, particularly at pH 6 or in double-stranded DNA for which the rearrangement of **7** into a spiro structure is less favored. This hypothesis awaits experimental evidence.

These examples show that whatever the modifications at C5, it is possible to observe structures of guanine oxidation products with a hydration at the C8 carbon without oxidation of this position (Scheme 22).

In summary, the products of guanine oxidation without an

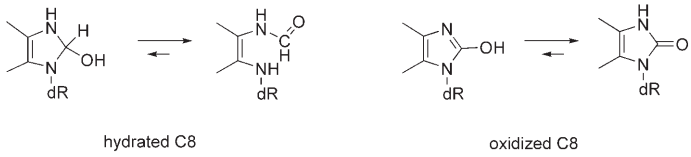


Scheme 20. Attack of a 5′-OH group at the C8 of **7**. The base is in an *anti* conformation. The O atom of the sugar is above the plane.



Scheme 21. Proposed products from **7**. RedSp was observed but not fully characterized; RedGh and RedIz are postulated.

oxidation at C8 are Iz (or **8** in the particular case of a possible intramolecular nucleophilic attack at C8) produced from intermediate **5**, or would be RedSp and the putative RedGh (or **9** from intramolecular process) resulting from the key intermediate **7**. The products of guanine oxidation with an oxidized C8 position (or the products of 8-oxo-G oxidation) are DGh, from the key intermediate **4**, or Sp and Gh from the other key intermediate **3** (Scheme 23).



Scheme 22. C8 hydrated and C8 oxidized.

The four key intermediates **3**, **4**, **5**, **7** described in Scheme 23 are at the origin of all oxidation products of guanine described in this review. They have been generated either from radical chemistry (combination of $O_2^{\bullet-}$ with a nucleic acid base radical) or from nucleophilic chemistry (addition of a nucleophile H_2O, ROOH on a cationic intermediate of the nucleic acid base).

One-electron reactions with G lead to hydroperoxide intermediates **5** or **1**, whereas two-electron reactions lead to hydroxylated intermediate **7** or to 8-oxo-G as highlighted by the boxes in Scheme 23. The formation of **1** in the case of an electron-transfer process is not documented, but cannot be ruled out. Intermediate **1** (observed in the case of G oxidation with $^1O_2$) is unstable and is transformed into **2**, which immediately gives rise to **3** or **4** by the reaction of nucleophiles like H_2O or ROOH, respectively, at C5.

The one-electron oxidation of 8-oxo-G induces the formation of **4** by reaction of $O_2^{\bullet-}$ at C5. The two-electron oxidation of 8-oxo-G leads to **3** through the attack of a water molecule at C5, but can also lead to **4** by the attack of a nucleophilic peroxide at C5.

Additionally, intermediates **5** and **1** can also be produced from two-electron reactions when the nucleophilic attack of a water molecule as at C5 is replaced by the attack by peroxide. The example, in this review was the trapping of cationic species by $KHSO_5$ leading to **6**, that is, an analogue of **5**, or to **2** if $KHSO_5$ attacks at C5 (minor pathway). Inversely, in the presence of reductant, initial intermediate **1** can be reduced to 8-oxo-G. The question of the possible formation of intermediate **7** by one-electron reaction should be addressed.

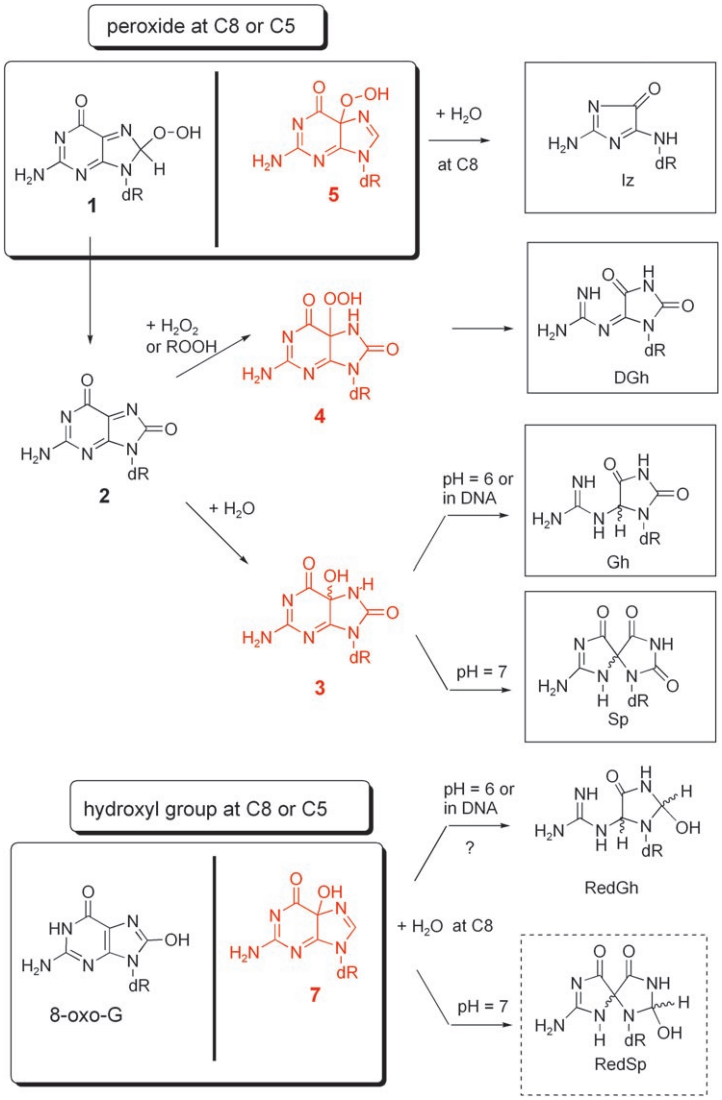
The products of guanine oxidation that derive from these key intermediates (**3**, **4**, **5**, and **7**) are shown in boxes for those that have been fully characterized (mass spectrometry and NMR analyses) and in dashed boxes for those that have not yet been characterized by NMR methods. The products not included in any box are postulated, but have not been observed.

Some of the products described in Scheme 23 are not stable. They may be detected after being hydrolyzed. The hydrolysis of Iz leads to Z,[6,59] the hydrolysis of DGh leads to Oa,[13,23] and can lead to Ua upon heating or at pH > 7.[22,60]

The trapping of transient oxidized species of G or 8-oxo-G by nitrogen-containing species (RNH_2, N-oxides) is not represented in Scheme 23 but follows the same logic. Examples of other radical traps ($NO_2^{\bullet}$, $CO_3^{\bullet-}$),[18,19,47] or other nucleophiles ($OONO^-$, amines,···) have already been described.[19,57,58,60–65] These mechanisms are relevant not only to guanine lesions but also to DNA-protein cross-links.

Conclusion

As can be seen from the studies of guanine oxidation by $HO^{\bullet}$, $^1O_2$, or electron transfer, the incorporation of an oxygen atom occurs either at C8 and/or C5 of G in the form of a hydroperoxide or a hydroxyl substituent. The final products of guanine oxidation, namely guanine lesions, arise from four possible key intermediate species **3**, **4**, **5**, and **7**. The formation of these intermediates as well as the subsequent reactions leading to guanine oxidation products depend on the experimental conditions (pH, presence of reducing agents, of radical traps, and so forth). FapyG and 8-oxo-G are the only oxidized guanine lesions that have been detected in vivo.[66] However, it seems reasonable to consider that all the guanine oxidation products described here are candidates for in vivo lesions. The guanine oxidation products formed by one-electron transfer followed by quenching



Scheme 23. Guanine oxidation products from intermediates **3**, **4**, **5**, and **7** at pH < 8. Products in boxes have been characterized by mass spectrometry and NMR analysis; the product in dashed box has been only characterized by mass spectrometry. Some products are postulated (not yet observed; no box).

of the guanine radical by $O_2^{\bullet-}$ and arising from the intermediate **5** (and perhaps to a lower extend from the intermediate **1**) are certainly biologically relevant. Intermediate **1** is also the key intermediate generated by $^1O_2$.The direct two-electron transfer mechanism (intermediate **7**) is less likely in vivo, since it needs strong oxidizing conditions. However, products derived from **7** should not be neglected, since they might also be formed from unexplored one-electron reactions like trapping of guanine radical by HO$^{\bullet}$. The products described from secondary oxidation of 8-oxo-G (from **3** or **4**) may be formed in the case of long-range electron transfer given that 8-oxo-G reacts as a hole sink. A first attempt to detect these new guanine oxidation products in vivo has been recently published, and Sp has been detected.[67]

Whatever the reactions taking place in vivo that probably elude straightforward clarification, the interest of in vitro studies on guanine oxidation with various oxidants provides the opportunity to analyze different possible G oxidation products and give a better idea of the possible fate of a guanine radical cation in cellular DNA. The study of the biological consequences of these probable G lesions is worthwhile.[60,68–80]

Acknowledgements

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