

Oxidatively Generated Damage to the Guanine Moiety of DNA: Mechanistic Aspects and Formation in Cells

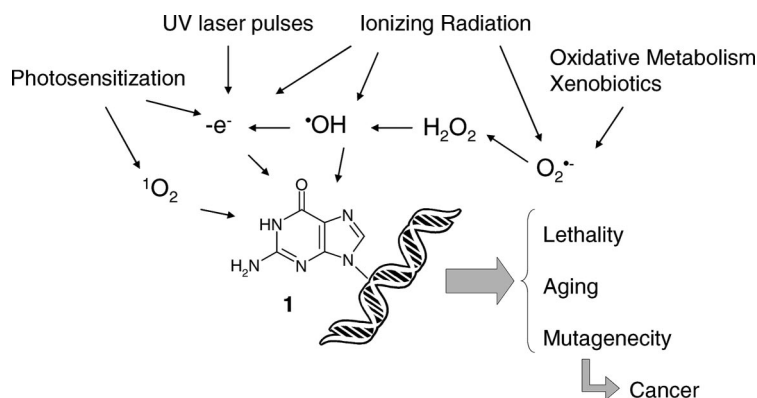
JEAN CADET,^{*,†,‡} THIERRY DOUKI,[†] AND JEAN-LUC RAVANAT[†]

[†]Laboratoire "Lésions des Acides Nucléiques", SCIB-UMR-E n3 (CEA/UJF) Institut Nanosciences et Cryogénie, CEA/Grenoble, F-38054 Grenoble Cedex 9, France,

[‡]Department of Nuclear Medicine and Health Science, University of Sherbrooke, Quebec J1H 5N4, Canada

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CONSPECTUS



Nuclear DNA and other molecules in living systems are continuously exposed to endogenously generated oxygen species. Such species range from the unreactive superoxide radical ($O_2^{\bullet-}$)—the precursor of hydrogen peroxide (H_2O_2)—to the highly reactive hydroxyl radical ($\bullet OH$). Exogenous chemical and physical agents, such as ionizing radiation and the UVA component of solar light, can also oxidatively damage both the bases and the 2-deoxyribose moieties of cellular DNA.

Over the last two decades, researchers have made major progress in understanding the oxidation degradation pathways of DNA that are most likely to occur from either oxidative metabolism or exposure to various exogenous agents. In the first part of this Account, we describe the mechanistic features of one-electron oxidation reactions of the guanine base in isolated DNA and related model compounds. These reactions illustrate the complexity of the various degradation pathways involved. Then, we briefly survey the analytical methods that can detect low amounts of oxidized bases and nucleosides in cells as they are formed. Recent data on the formation of oxidized guanine residues in cellular DNA following exposure to UVA light, ionizing radiation, and high-intensity UV pulses are also provided. We discuss these chemical reactions in the context of $\bullet OH$ radical, singlet oxygen, and two-quantum photoionization processes.

1. Introduction

Nuclear DNA as other biomolecules of living systems is continuously exposed to endogenously generated oxygen species such as the unreactive superoxide radical ($O_2^{\bullet-}$) that is the precursor of hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($\bullet OH$). The bases, the 2-deoxyri-

bose moieties, or both of cellular DNA may also be oxidatively damaged by a large number of exogenous chemical and physical agents including ionizing radiation and the UVA component of solar light. Major efforts have been devoted during the last two decades to the elucidation of oxidative pathways mediated by 1O_2 , $\bullet OH$, and one-electron oxidants using nucleobases, nucleosides,

and oligonucleotides as DNA model compounds. This has led to the isolation and characterization of more than 70 modified nucleosides if diastereomers and relatively unstable initial oxidation products such as thymidine hydroperoxides are included.¹ It may be added that relevant information on structural and chemical features of radical precursors of most of the oxidized nucleobases has been inferred from electron spin resonance, laser flash photolysis, and pulse radiolysis studies.^{1e,2} As a result, comprehensive mechanisms are available for most of the oxidation reactions of purine and pyrimidine DNA bases.¹

Emphasis is placed in the first part of this Account on recent mechanistic aspects of one-electron oxidation reactions of the guanine (**1**) moiety of DNA fragments for which a large consensus now exists.³ Prior to providing insights into several oxidation pathways of guanine (**1**) in cellular DNA mediated by photosensitized ¹O₂, radiation-induced •OH, and two-quantum photoionization, a brief survey of the analytical methods aimed at singling out the formation of low amounts of oxidized bases and nucleosides in cells is reported.

2. One-Electron Oxidation Reactions of the Guanine Moiety of Isolated DNA and Model Systems

Numerous chemical and physical agents have the ability to abstract one electron from guanine, which exhibits the lowest ionization potential among DNA components, explaining why guanine (**1**) has been found to be the predominant sink for hole transfer in double-stranded DNA. Thus several agents including biologically relevant nitrosoperoxycarbonate, the product of the reaction of peroxyxynitrite with carbon dioxide, have been shown to promote one-electron oxidation of **1** as part of either free nucleoside or isolated DNA.⁴ In addition, ionizing radiation through the direct interaction of highly energetic photons with DNA, triplet-excited type I photosensitizers, and high-intensity UVC laser pulses are able to efficiently oxidize guanine (**1**). Evidence was gained, mostly from pulse radiolysis experiments on transient radicals using the redox titration technique and the characterization of the final oxidation products, that the guanine radical cation (**2**) thus generated may undergo in aqueous solutions two main competitive pathways, namely, nucleophilic addition and deprotonation reactions.

2.1. Nucleophilic Addition Reactions to the Guanine Radical Cation (2). The first experimental proof for the occurrence of a nucleophilic addition to the guanine radical cation (**2**) was provided by the observation of the incorporation of an

¹⁸O-atom in 8-oxo-7,8-dihydroguanine (**4**), which was generated as a major degradation product upon riboflavin photosensitized oxidation of calf thymus DNA in aerated [¹⁸O]-labeled water solutions.⁵ The hydration reaction, whose pseudo-first-order rate constant has been estimated to be $6 \times 10^4 \text{ s}^{-1}$ in double-stranded DNA,⁶ gives rise to the reducing 8-hydroxy-7,8-dihydroguanyl radical (**3**) (Figure 1).² A counterion-assisted proton shuttle mechanism has been proposed on the basis of molecular dynamics and ab initio quantum simulations for the water molecule addition at C8 of **2** in a DNA duplex.⁷ Radical **3** can be also generated by •OH addition at the C8 of **1** according to a reaction that was estimated to take place in a 17% yield with the free nucleoside.⁸ Oxidation of the latter radical as the result of fast O₂ reaction that occurs with a rate constant of $4 \times 10^9 \text{ s}^{-1}$ leads to the formation of **4**, whereas competitive reduction, which is predominant in oxygen-free aqueous solution, gives rise to 2,6-diamino-4-hydroxy-5-formamidopyrimidine (**5**).⁸ This involves the opening of the imidazole ring at the C8–N9 bond (Figure 1) with a rate constant ($k = 2 \times 10^{-5} \text{ s}^{-1}$) that has been estimated by pulse radiolysis.⁸

Further evidence for the major role played by nucleophilic addition at C8 in the reactions of **2** was gained from the isolation and characterization of the cross-link formed upon photoexcited riboflavin-mediated one-electron oxidation of TGT trinucleotide in the presence of the KKK trilycine peptide (Figure 1).⁹ It was found that the free amino group of the central lysine residue of KKK peptide was able to covalently attach to the C8 carbon of the guanine residue of the trinucleotide in a highly efficient way that prevents the competitive formation of **4**. The 8-substituted guanine adduct **6** thus formed was shown to be highly susceptible to further one-electron oxidation, which, as previously observed for **4**,^{10,11} gives rise to spiroiminodihydantoin compounds through an acyl shift rearrangement of a transiently generated 5-hydroxyl adduct. The nucleophilic addition of a lysine residue to **2** is likely to explain the observed formation of cross-links between double-stranded DNA and proteins including histones upon specific one-electron oxidation of the guanine bases.¹² As an alternative mechanism, the formation of the cross-link **6** upon riboflavin-mediated photosensitization of an aerated aqueous solution of d(ATGC) tetranucleotide and N^α-acetyllysine has been recently proposed to involve initial generation of lysine aminium radical cation that is able to add to C8 of the guanine moiety.¹³ Another example of nucleophilic addition at the C8 of **1** upon one-electron abstraction by CO₃^{•-} radical ions and other one-electron oxidants in a single-stranded oligonucleotide has recently become available. Thus it was

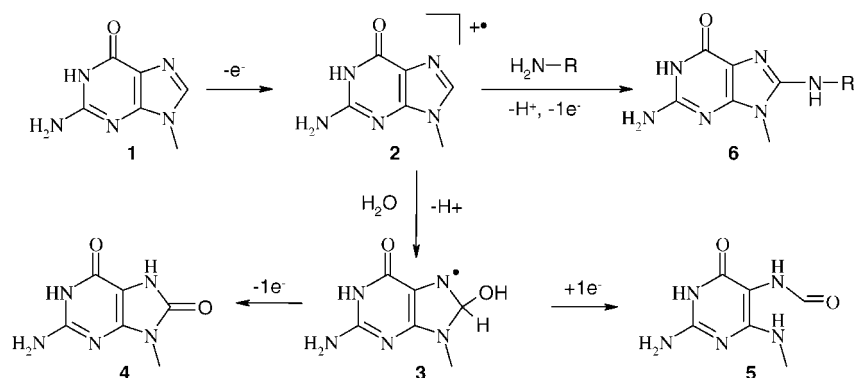


FIGURE 1. Nucleophilic reactions of the guanine radical cation (**2**) at C8.

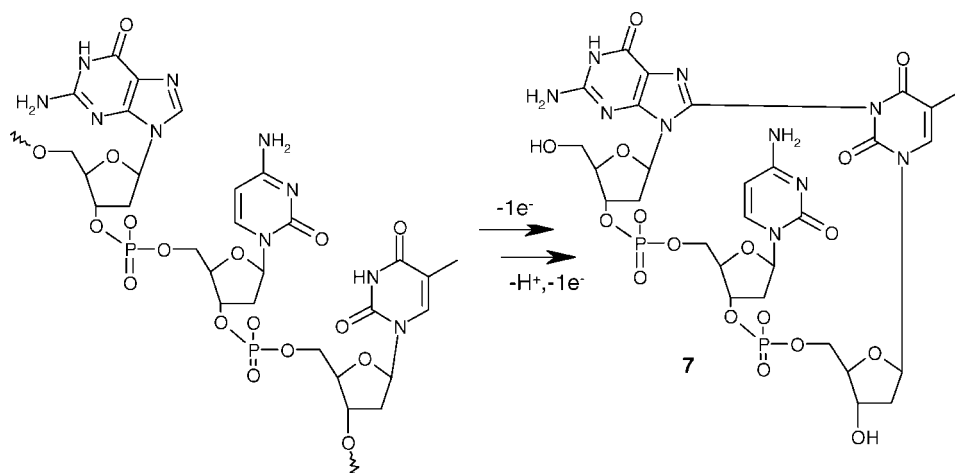


FIGURE 2. Structure of a thymine-guanine cross-link.

shown that a distant thymine base, which is separated from **2** or its deprotonated form **8** by a cytosine 2'-deoxyribonucleotide, is able to covalently bind through its N3 atom to the guanine base giving rise to the intrastrand cross-link **7** (Figure 2).¹⁴

2.2. Deprotonation Pathway Involving the Formation of the Highly Oxidizing G(-H)[•] radical **8.** Deprotonation of the base radical cation **2** of 2'-deoxyguanosine, which exhibits a pK_a value of 3.9,² occurs at N1 with a rate constant¹⁵ of $1.8 \times 10^7 \text{ s}^{-1}$ leading to the formation of the highly oxidizing radical **8** (Figure 3). A similar fast deprotonation reaction occurs in G, GG, or GGG containing double-stranded DNA according to a fast and slower decay rate constants that have been estimated to be $1.3 \times 10^7 \text{ s}^{-1}$ and $3 \times 10^6 \text{ s}^{-1}$, respectively.¹⁵ The neutral guanine radical **8** may also be generated by efficient dehydration of the overwhelming [•]OH radical adduct at C4 of **1** with a rate constant of $6 \times 10^3 \text{ s}^{-1}$ at neutral pH.⁸ Recently, information on the assignment and properties of two G(-H)[•] tautomers that were produced by protonation of 8-bromo-2'-deoxyguanosine electron adduct was gained from comprehensive pulse radiolysis and DFT studies.¹⁶

Evidence has been provided that, at best, O_2 would react very slowly ($k < 10^3 \text{ M}^{-1} \text{ s}^{-1}$) with **8**,¹⁷ as also observed for oxidizing radicals derived from tryptophan and tyrosine. In contrast, **8** is able to efficiently quench superoxide anion radical ($\text{O}_2^{\bullet-}$) with rate constants of $3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and $4.7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ for nucleosides⁸ and DNA duplex respectively.¹⁷ Addition of $\text{O}_2^{\bullet-}$ to C-5 carbon-centered radical **9**, one of the possible resonance forms of **8**, is the first step of a rather complicated decomposition pathway leading to the formation of 2,2,4-triamino-5(2H)-oxazolone (**14**) as the main end-product (Figure 3).¹⁸ This may be rationalized in terms of formation of a 5-hydroperoxide **10** by radical combination of $\text{O}_2^{\bullet-}$ with G(-H)[•] followed by protonation. Subsequent nucleophilic addition of a water molecule across the 7,8-double bond of **10** leading to **11** is followed by opening of the pyrimidine ring at C5-C6 bond and decarboxylation. Further rearrangement of **12** thus produced involves the release of a formamide molecule through ring-chain tautomerism of the carbinolamine function¹⁹ before cyclization that gives rise to 2,5-diamino-4H-imidazol-4-one (**13**). Hydrolysis of **13**, whose half-life is about 10 h in neutral aqueous solutions at 20 °C, leads to

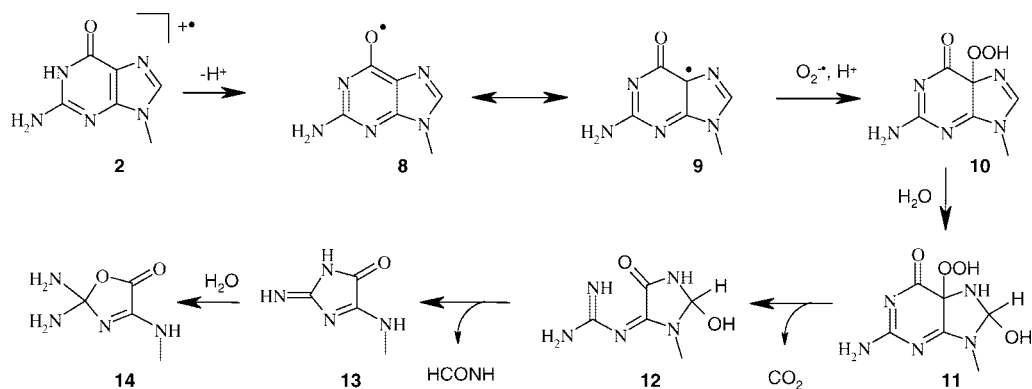


FIGURE 3. Reactions of the guanine oxidizing radical **8**.

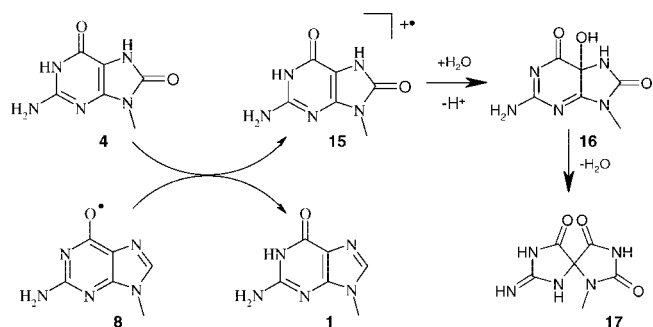


FIGURE 4. Repair of guanine radical cation (**2**) by 8-oxo-7,8-dihydroguanine (**4**).

the quantitative formation of **14**. Confirmation of the occurrence of a nucleophilic reaction during the fate of **8** was provided by the observation of intramolecular additions at C8 involving either the 5'-hydroxymethyl group of free 2'-deoxyguanosine^{1a} or a tethered lysine residue at the 5' end of modified nucleosides.²⁰ A major competitive reaction of **8** in isolated nucleosides involves efficient oxidation of **4** that is consumed as soon as it is generated²¹ (Figure 4) leading to the formation of spiroiminodihydroantoin (**17**)²² and **13**.¹⁸ This may be related to the high values of the rate constants of the reaction between **8** and **4** as either the 2'-deoxyribonucleoside²³ or the corresponding 5'-phosphomonoester derivative²⁴ that have been found to be $4.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $9.3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, respectively. However the efficiency of intrastrand oxidation of **4** by **8** appears to be much lower,¹⁷ making the putative protecting role of easily oxidizable sites against oxidative processes through hole transfer unlikely in cellular DNA.²⁵ Reduction of **8** by electron transfer that leads to restoration of the guanine residue (**1**) has been proposed to be a competitive reaction mediated by $\text{O}_2^{\bullet -}$ in order to explain the poor formation efficiency of **13** in DNA duplexes upon one-electron oxidation.¹⁷

3. Guanine Oxidation Reactions of Cellular DNA

3.1. Methods of Measurement. The measurement of oxidized bases and nucleosides in cellular DNA may be used to gain insights into the nature and importance of chemical reactions that are generated in cellular DNA by oxidizing agents. For this purpose, targeted modified bases or nucleosides are usually separated by a chromatographic method from the overwhelming normal DNA components after a suitable hydrolytic or enzymatic digestion step. The detection of compounds of interest at the output of the column requires a sensitive technique that should be able to single out a few lesions per 10^6 – 10^7 nucleosides in a DNA sample size of about 20 to 30 μg .^{1b} However this has been hampered until recently by the use of inappropriate methods that have led in most cases to overestimated values of the levels of DNA oxidized bases by factors varying from 1 to 3 orders of magnitude.^{1b} The origin of the main drawbacks that were associated with the use of the questionable gas chromatography–mass spectrometry (GC–MS) method,²⁶ introduced more than 20 years ago, is now identified.^{1b} Thus, spurious oxidation of the normal bases has been shown to occur with an efficiency of about 0.1% during the derivatization step that is required to make the samples volatile.²⁷ This has led to the artifactual generation of oxidized purine and pyrimidine bases such as 8-oxo-7,8-dihydroguanine, 8-oxo-7,8-dihydroadenine, and 5-(hydroxymethyl)uracil preventing any accurate measurement to be made. A second matter of concern that is shared by the chromatographic assays requiring an acidic hydrolysis step for the release of the bases is the lack of stability of several modifications including formamidopyrimidine derivatives of adenine and guanine under hot acid formic treatment.²⁸ A third source of artifacts, although usually of lower amplitude, that may occur for all HPLC and GC assays deals with adventitious Fenton-type oxidation reactions during the DNA extraction

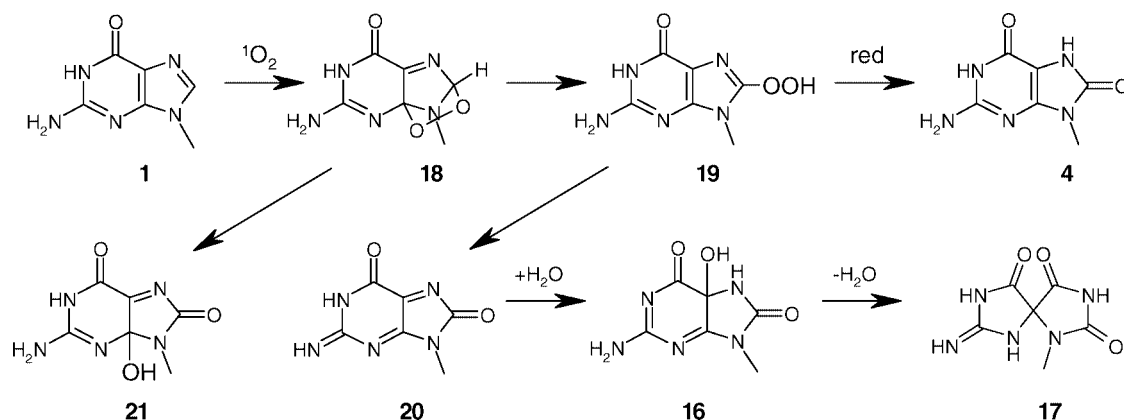


FIGURE 5. Singlet oxygen reactions of the guanine moiety of nucleosides and DNA.

and digestion steps.^{1b} A general consensus now exists on improved chromatographic methods aimed at measuring 8-oxo-7,8-dihydro-2'-deoxyguanosine (**4**) through the cooperative efforts of the European Standard Committee on Oxidative DNA Damage (ESCODD) network that has involved 25 laboratories.²⁹ Recommended protocols that include suitable conditions of DNA extraction for which artifactual oxidation is minimized followed by suitable high-performance liquid chromatography analysis of the DNA digest are now available.³⁰ The frequently used electrochemical detection technique (HPLC–ECD), which was introduced more than 20 years ago,³¹ is a robust method whose application in the oxidative detection mode is, however, restricted to only a few electroactive DNA lesions including **4**, 8-oxo-7,8-dihydro-2'-deoxyadenosine (**25**), and 5-hydroxy-substituted pyrimidine nucleosides.³² The recently available electrospray ionization–tandem mass spectrometry (MS/MS) method³³ operating in the multiple reaction monitoring mode is more versatile and, on the average, more sensitive than HPLC–ECD, allowing the accurate measurement of up to 15 base modifications in cellular DNA among the 70 identified so far in model compounds. Accurate determination of very low amounts of radiation-induced guanine[8–5]cytosine intrastrand cross-link (0.037 lesions per 10^9 normal bases and per Gy) has been made possible by the use of HPLC-MS³ analysis.^{33c}

3.2. Singlet Oxygen Reactions. A suitably protected naphthalene endoperoxide that can penetrate cells has been used to investigate the $^1\text{O}_2$ oxidation of nuclear DNA.³⁴ Thus, the release of $^1\text{O}_2$ from the thermolabile endoperoxide precursor led to the selective oxidation of **1** by producing exclusively **4** as measured by HPLC–MS/MS.³⁵ As a relevant piece of information, it was established that the formation of **4** in cellular DNA was due to singlet oxygen and not to a putative oxidative stress. This was supported by labeling experiments involving a synthetically prepared [$^{18}\text{O}_2$]-endoperoxide.

The formation of **4** in cellular DNA is accounted for by initial Diels–Alder [4 + 2] cycloaddition of $^1\text{O}_2$ across the imidazole ring of **1** leading to the generation of a pair of diastereomeric 4,8-endoperoxides **18** before rearrangement into 8-hydroperoxyguanine (**19**) and reduction as proposed from model studies (Figure 5).³⁶ The competitive dehydration of **19** that is a predominant pathway for isolated nucleoside giving rise to the two diastereomers of **17**³⁷ through a highly reactive quinonoid intermediate **20**³⁸ appears to be at best a minor pathway in cellular DNA under mild conditions of oxidation. This also applies to the diastereomers of 4-hydroxy-8-oxo-7,8-dihydro-2'-deoxyguanosine (**21**) that have been recently shown to be generated as minor products of $^1\text{O}_2$ oxidation of 2'-deoxyguanosine (**1**),³⁹ whereas **17** is predominant under these conditions.^{37a} It has also been found that $^1\text{O}_2$ is not able to induce significant amounts of direct DNA strand breaks or alkali-labile sites as inferred from comet assay measurements.⁴⁰ This is also indicative of a very low formation, if any, of **17**, which is known to be alkali-labile. It is now well documented that exposure to UVA irradiation, a major component of solar light, is able to generate **4** in mammalian and bacterial cells⁴¹ and also in human skin.⁴² A detailed mechanistic study performed on human monocytes, which has involved a comparison with the effects of radiation-induced $\cdot\text{OH}$ radical, was performed using a modified version of the alkaline comet assay (Table 1). The yields of γ -radiation-induced modified purine residues and oxidized pyrimidine bases whose formation arise mostly from $\cdot\text{OH}$ reactions were assessed as formamidopyrimidine (Fpg)- and endonuclease III (endo III)-sensitive sites, respectively. The ratio of the lesions recognized and processed by the two latter repair enzymes was 1 to 1 whereas the sum of the strand breaks (ssb) and alkali-labile sites (als) was found to be 2.3-fold higher than any of the two classes of modified bases. The situation is strongly different for UVA effects. The ratio between strand

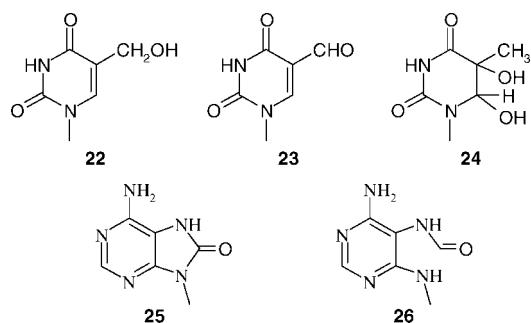
TABLE 1. UVA-Induced Damage^a to Cellular DNA^{b,c}

classes of lesions	control	γ rays (yield/Gy)	UVA radiation (yield/(kJ·m ⁻²))
Fpg-sensitive sites	190	48	1.9
endo III-sensitive sites	195	53	0.3
strand breaks	265	130	0.9

^a DNA modifications revealed as strand breaks using the comet assay. ^b THP-1 malignant cells. ^c Table adapted from refs 1b and 43.

breaks (ssb + als) and oxidized pyrimidine bases is still close to three whereas the formation of Fpg-sensitive sites is about 6-fold more important than that of oxidized pyrimidine bases. This strongly suggests that about 80% of the UVA-photosensitized formation of **4** in DNA was due to ¹O₂ oxidation as the result of type II photosensitization mechanism.⁴³ A Fenton-type radical mechanism, which would involve initial generation of superoxide radical followed by its spontaneous or enzymic dismutation into H₂O₂, would explain the formation of oxidized pyrimidine bases and 20% remaining **4**.⁴³

3.3. •OH-Mediated Degradation Pathways. Ionizing radiation constitutes a suitable way to generate both •OH and one-electron oxidation events in cells. Several classes of radical degradation products of thymidine, 2'-deoxyguanosine, and 2'-deoxyadenosine that were previously characterized in model studies^{1a} were detected in the DNA of γ -irradiated THP-1 human monocytes by HPLC–ECD and HPLC–MS/MS measurements⁴⁴ in the isotope dilution mode.⁴³ These consisted of six thymidine oxidation products and four purine lesions whose formation was linear with the applied doses (0–100 Gy) of low linear energy transfer (LET) γ -rays. 5-(Hydroxymethyl)-2'-deoxyuridine (**22**) and 5-formyl-2'-deoxyuridine (**23**) represent two methyl oxidation products, whereas the four *cis* and *trans* diastereomers of 5,6-dihydroxy-5,6-dihydrothymidine (**24**) (Figure 6) arise from radical reactions involving the 5,6-ethylenic bond.^{1a} The two main radiation-induced degradation products of the purine bases were identified as **4** and **5**, whereas the two related adenine degradation products, namely, 4,6-diamino-5-formamidopyrimidine (**26**) and **25** (Figure 6) are generated with an ~10-fold lower efficiency. The radiation-induced formation yield of the various oxidized nucleosides is comprised between 1 and 97 lesions per 10⁹ bases and Gy (Table 2). This has to be compared with previous questionable measurements achieved either by GC-MS⁴⁵ or more recently by HPLC–MS⁴⁶ that were between 2 and 3 orders of magnitude higher. A similar product distribution was observed upon exposure of cellular DNA to highly energetic ¹²C⁶⁺ and ³⁶Ag¹⁸⁺ heavy ions.⁴⁴ The formation of the oxidation products may be depicted by the predominant implication of •OH arising from the indirect effect of

**FIGURE 6.** Oxidation products of thymine and adenine in cellular DNA.**TABLE 2.** Radiation-Induced Base Damage^a to Cellular DNA^{b,d}

lesions (number per Gy and 10 ⁹ bases)	γ rays	¹² C ⁶⁺ ions ^c
8-oxo-7,8-dihydro-2'-deoxyguanosine (4)	20	10
2,6-diamino-4-hydroxy-5-formamidopyrimidine (5)	39	22
5-(hydroxymethyl)-2'-deoxyuridine (22)	29	12
5-formyl-2'-deoxyuridine (23)	22	11
5,6-dihydroxy-5,6-dihydrothymidine (24)	97	62
8-oxo-7,8-dihydro-2'-deoxyadenosine (25)	3	3
4,6-diamino-5-formamidopyrimidine (26)	5	1

^a Determined by HPLC–MS/MS. ^b THP-1 malignant cells. ^c Linear energy transfer = 31.5 keV/ μ m. ^d Table adapted from refs 1b and 44a.

γ -rays or heavy particles, even if it is not possible to completely rule out a contribution of ionization reactions, which lead to similar degradation products. Support for the major role played by •OH came from the consideration of the effects of the radiation quality on the formation efficiency of oxidized nucleosides that was found to decrease with the increase in LET. This may be rationalized in terms of decrease in the yield of •OH due to higher recombination processes with LET increase. In addition, the existence of efficient charge transfer reactions that lead to the predominant formation of **4** following initial ionization of the pyrimidine and purine bases upon exposure to high-intensity UVC laser pulses (*vide infra*) is a second argument although indirect in favor of the predominant implication of •OH in the formation of base oxidation products. The formation of **4**, which is a ubiquitous oxidatively generated base damage, may be rationalized by initial addition of •OH at the C8 of the imidazole ring. Oxidation of the radical **3** thus formed leads to the formation of **4**, whereas predominant competitive one-electron reduction likely due to poorly oxygenated nucleus gives rise to **5** (Figure 1). Another example of •OH-mediated oxidation reactions of the guanine is provided by the HPLC–MS/MS detection of 2,2-diamino-4-[(2-deoxy- β -D-erythro-pentofuranosyl)amino]-5(2H)-oxazolone (**14**) in hepatic DNA of diabetic rats.⁴⁷

3.4. Two-quantum photoionization. Ionization processes of both the nucleobases and the 2-deoxyribose moieties are expected to be associated with the direct effect of γ

TABLE 3. One-Electron Oxidation Base Lesions^a in Cellular DNA^b upon Two-Photon UVC^c Photoionization^d

base lesions ^a	yield per 10 ⁶ bases and mJ
8-oxo-7,8-dihydro-2'-deoxyguanosine (4)	1.29
5-(hydroxymethyl)-2'-deoxyuridine (22)	0.06
5-formyl-2'-deoxyuridine (23)	0.02
5,6-dihydroxy-5,6-dihydrothymidine (24)	0.17

^a Determined by HPLC–MS/MS. ^b THP-1 malignant cells. ^c Laser pulses at 266 nm. ^d Table adapted from ref 44b.

rays. Two quantum photoionization provided by 266 nm nanosecond laser pulses has been shown to be an efficient way to oxidize purine and pyrimidine bases of free nucleosides and isolated DNA.^{3b} Depletion of the initially generated triplet excited-state nucleobases leads by absorption of a second UV photon to the generation of the related radical cations and subsequent chemical reactions. This approach has been successfully applied to investigate in a specific way the chemistry of purine and pyrimidine radical cations in cellular DNA on the basis of the HPLC–MS/MS measurement of dedicated final oxidation products.^{44b} Thus it was found that **4** was formed predominantly over one-electron oxidation products of thymidine including **22–24** (Table 3). The formation of the pyrimidine degradation products may be rationalized in terms of transient generation of thymine radical cation whose chemical reactions in aerated aqueous solutions have been assessed on the basis of model studies involving type I photosensitizers.^{3b} The formation of **4** is likely to result from hydration of **2** (Figure 1), which may be produced through direct one-electron oxidation of a guanine residue or subsequent to hole migration to a guanine base that acts as a sink from a distant adenine or pyrimidine radical cation. This is suggestive of occurrence of charge transfer reactions within cellular DNA that have been shown to take place within double-stranded oligonucleotides according to several mechanisms including multistep hopping, phonon-assisted polaron-like hopping, and coherent superexchange.⁴⁸

4. Summary and Outlook

Evidence is provided in this short survey on the formation of several modified bases in cellular DNA after exposure to physical and chemical oxidizing agents. This validates, at least partly, in living cells the mechanisms of degradation of nucleobases by ¹O₂, •OH, and one-electron oxidants that were previously inferred from model studies. This was achieved using in most cases the accurate and specific HPLC–MS/MS analytical technique that has also involved application of optimized conditions of DNA extraction protocols in order to minimize the occurrence of spurious oxidation. Emphasis should now be

placed on the detection of other guanine lesions such as DNA–protein cross-links.^{9,11}

BIOGRAPHICAL INFORMATION

Jean Cadet received his Ph.D. in chemistry from the University of Grenoble in 1973 and has been research associate and visiting professor in several institutions including John Hopkins University (1977 and 1980), Atomic Energy of Canada at Pinawa (1980), University of Manitoba at Winnipeg (1987), and Dublin City University (2006 to the present). He is currently Scientific Adviser at the French Atomic Energy Commission at CEA/Grenoble and Adjunct Professor at University of Sherbrooke. He is involved in research activities on various aspects of the chemistry and biochemistry of oxidatively generated and photoinduced damage to DNA (mechanisms of reactions, measurement in cells, assessment of biological features, such as substrate specificity of DNA repair enzymes, and mutagenesis of base lesions).

Thierry Douki received his Ph.D. degree in chemistry in 1992 from Grenoble University. After that, he joined Prof. Bruce Ames' group as a postdoctoral fellow in Berkeley. He was then hired by the French Atomic Energy Commission in Jean Cadet's laboratory in Grenoble. His scientific activity is devoted to the identification and the repair of DNA damage induced by solar UV light, ionizing radiation, oxidative stress, and chemicals.

Jean-Luc Ravanat was born in 1964 and studied biochemistry and organic chemistry at Universities of Montpellier and Grenoble, France. He obtained his Ph.D. in 1992 and then spent two years at Nestlé Research Centre in Switzerland. Since 1996, he has been a staff member of the laboratory and his work is focused on the identification of the nature and the mechanisms of formation of DNA lesions mediated by several carcinogens including reactive oxygen species. He has significantly contributed to the development of sensitive assays aimed at measuring DNA lesions at the cellular level.

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