Comparative study of base damage induced by gamma radiation and Fenton reaction in isolated DNA

Sandrine Frelon, Thierry Douki,* Alain Favier and Jean Cadet *

Laboratoire "Lésions des Acides Nucléiques", Service de Chimie Inorganique et Biologique, UMR 5046, CEA/DSM/Département de Recherche Fondamentale sur la Matière Condensée, CEA-Grenoble, 38054 Grenoble Cedex 9, France

Received (in Cambridge, UK) 1st August 2002, Accepted 9th October 2002
First published as an Advance Article on the web 8th November 2002

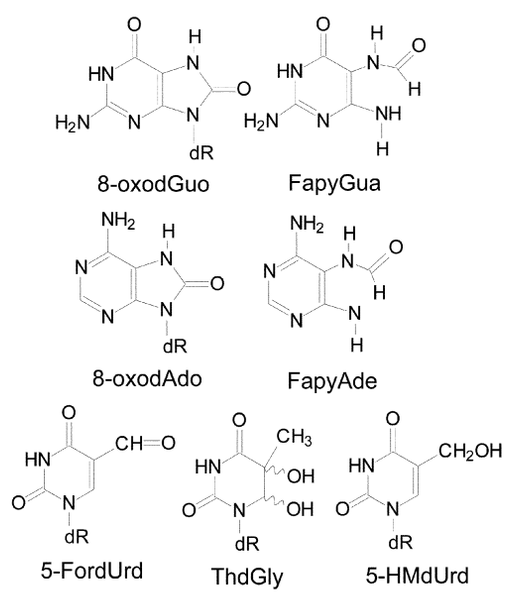
Both γ-irradiation and Fenton reaction generate oxidising species that damage isolated DNA. The comparison of the distribution of oxidised bases suggested that the Fenton reaction produces hydroxyl radicals like ionizing radiation. However, the former process was also found to generate a reducing species that increased the yield of formamidopyrimidine derivatives at the expense of the 8-oxo compounds of purine bases. Another peculiarity of the Fenton reaction was its ability to induce the formation, though in very low yield, of the malonaldehyde-2′-deoxyguanosine adduct in DNA, likely *via* the formation of base propenals upon degradation of the 2-deoxyribose unit.

Introduction

Degradation of DNA by oxygen radicals is an important genotoxic mechanism associated with ageing and carcinogenesis.[1] A wide range of oxidising species are produced by cellular metabolism as well as by environmental stresses such as radiation and chemicals. Identification of DNA degradation pathways associated with specific deleterious oxidative species is required for a better understanding of pathological processes related to oxidative stress. In cells, highly reactive species, such as hydroxyl radicals (˙OH), may be produced through the reduction of hydrogen peroxide, the dismutation product of superoxide anion,[2] by ferrous ions. This process, known as the Fenton reaction, becomes cyclic in the presence of a reducing species able to reduce the resulting Fe^{3+} into Fe^{2+}. When the reducing species is superoxide anion, the iron-catalysed conversion of hydrogen peroxide is referred to as the "Haber–Weiss cycle". To prevent the deleterious effect of the latter processes, iron storage is strictly controlled within cells,[3] for instance through its sequestration by ferritin. However, the cytotoxic potential of iron may be expressed under some pathological conditions such as inherited hemochromatosis and iron overload. Iron release has also been observed under oxidative stress conditions, for instance UVA irradiation.[4] Altogether, the iron-catalysed production of reactive oxygen species is likely to damage key biomolecules including DNA, and thereby to be associated with genotoxicity.[5,6] However, the identity of the oxidising species produced during the Fenton reaction, in particular the actual formation of ˙OH, is still a matter of debate.[7–9]

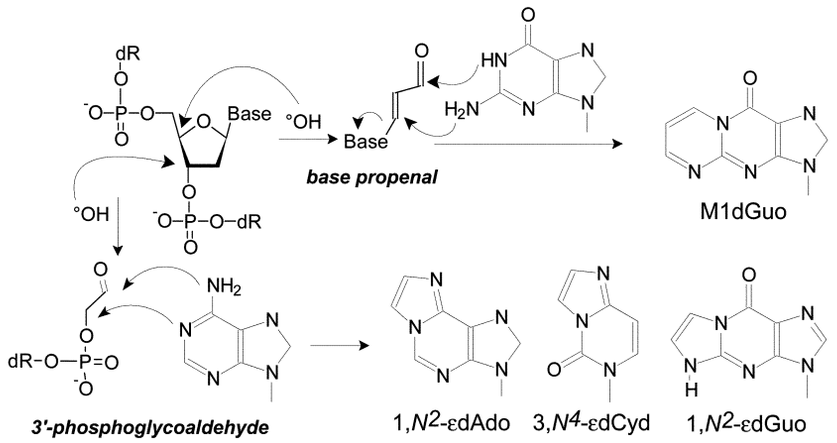
The extensively studied decomposition of water by ionizing radiation provides another source of ˙OH radicals. The DNA bases degradation reactions induced under these conditions have been widely investigated, in terms of formation of primary nucleobase radicals[10] and identification of the final products.[11] The comparison between the relatively well understood γ-radiolysis of DNA and the damage produced in DNA under the conditions of Fenton reaction may shed some light on the actual oxidising species involved in the latter process. The few extensive studies on the degradation of free nucleosides under Fenton reaction conditions have revealed some differences with respect to γ-radiation.[12,13] A reliable comparison between the radical-induced damage to isolated DNA under the two latter conditions is still needed and is likely to provide relevant mechanistic information. Indeed, the distribution of base damage within DNA has been shown to be very sensitive to the reaction conditions, such as the redox conditions and the presence of oxygen.[11] A relevant example is provided by the competitive pathways involved in the ˙OH-induced degradation of 2′-deoxyguanosine.[14–16] Indeed, hydroxyl radicals may add to the aglycone of the latter nucleoside at either the C4 or the C8 position.[10] In the latter case, the resulting neutral reducing radical may be either oxidised into 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) or reduced into 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) nucleoside.

Using a specific and sensitive high performance liquid chromatography–tandem mass spectrometry assay, we undertook a comparative study of the formation of radical-induced base damage (Scheme 1) produced upon either gamma irradi-



Scheme 1 Chemical structure of the quantified radical-induced DNA base damage.

 DOI: 10.1039/b207532f



Scheme 2 Chemical structure and proposed mechanism of formation of the 2-deoxyribose degradation mediated-base damage.

ation or exposure to Fenton reaction conditions. In the latter experiments, complexes of ferrous ions were used, instead of ferric salts in the presence of reducing agents. Indeed, the latter compounds may modify the chemical conversion of DNA base radicals. In addition, in order to determine a possible role of metal ions in the $^{\bullet}OH$-induced degradation of DNA, gamma irradiations were also carried out in the presence of iron. It was thus possible to reliably compare the effects of the reactive species produced upon radiolysis of water on one hand and Fenton reaction on the other hand. The level of exocyclic adducts, including 3,N^4-ethenocytosine, 1,N^2-ethenoguanine, 1,N^2-ethenoadenine and the pyrimidinopurine malonaldehyde guanine adduct, proposed to arise from the reaction of DNA bases with 2-deoxyribose degradation products (Scheme 2),[17–19] was also measured.

Experimental

Chemicals

Nuclease P1 (*Penicillium citrium*), calf spleen phosphodiesterase I and II, and calf thymus DNA were obtained from Sigma (St Louis, MO). Alkaline phosphatase was purchased from Roche Diagnostics (Mannheim, Germany). Ethylenediaminetetraacetic acid (EDTA) was from Interchim (Montluçon, France). Water was deionized with a Millipore-Milli-Q system (Millipore, Molsheim, France). M1dGuo was prepared by enzymatic 2-deoxyribosylation of the guanine derivative.[20] 1,N^2-Etheno-2′-deoxyadenosine (1,N^2-εdAdo), 3,N^4-etheno-2′-deoxycytidine (3,N^4-εdCyd) and 1,N^2-etheno-2′-deoxyguanosine (1,N^2-εdGuo) were prepared by incubation of the corresponding nucleosides with chloroacetaldehyde. The four latter cyclic adducts were purified by HPLC and characterised by mass spectrometry and ^{1}H NMR. In all cases, spectroscopic features were identical to published data. Calibrated solutions were prepared by UV spectrophotometry using available molecular absorption coefficients.

γ-Irradiation of DNA in aerated aqueous solutions

An aerated aqueous solution of DNA (0.5 mg ml^{-1}) to which either $FeSO_4$ or $FeCl_3$ had been added (final concentration 0 to 100 μM in the presence of equimolar concentration of EDTA), was exposed to the γ-rays of a ^{60}Co source immersed in a water pool. The dose rate was 20 Gy min^{-1} as determined by poly(methylmethacrylate) dosimetry. Air was continuously bubbled through the solution during the irradiation period (2 to 5 min).

Oxidation of DNA under Fenton chemistry conditions

An aerated aqueous solution of DNA (0.5 mg ml^{-1}) was treated with freshly prepared ferrous sulfate solutions (0–100 μM final concentration) in the presence or the absence of equimolar amount of EDTA. Hydrogen peroxide was then added (final concentration: 0–200 μM). The reaction mixture was kept at 37 °C for 1 h. DNA was then precipitated and solubilised in a 0.1 mM desferroxamine mesylate solution. Studies on the formation of exocyclic adducts were performed in a similar way with 500 μM H_2O_2 and 100 μM $FeSO_4$. EDTA (0–500 μM) was added in some experiments.

Measurement of modified bases within DNA

Following oxidation, DNA was precipitated and digested into nucleosides as previously described[21] by incubation with phosphodiesterases I and II, nuclease P1 and alkaline phosphatase. The samples were then analysed by reverse phase HPLC associated with an API 3000 mass spectrometer (Perkin-Elmer/SCIEX, Thornhill, Canada) used in the multiple reaction monitoring mode. Oxidised nucleosides quantified by isotopic dilution[21] included 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyadenosine (8-oxodAdo), 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd), 5-formyl-2′-deoxyuridine (5-FordUrd) and the four *cis* and *trans* diastereoisomers of 5,6-dihydroxy-5,6-dihydrothymidine (thymidine glycols, ThdGly). 1,N^2-εdAdo, 3,N^4-εdCyd, 1,N^2-εdGuo and M1dGuo were quantified by HPLC-MS/MS in the positive mode. For the four cyclic adducts, the monitored transitions corresponded to the loss of the 2-deoxyribose moiety as reported by others.[22,23] An external calibration of the HPLC-MS/MS detector was performed for the latter lesions. In each sample, the amount of DNA injected was inferred from the area of the peak corresponding to 2′-deoxyguanosine monitored in a UV spectrophotometer (λ: 280 nm) placed prior to the inlet of the mass spectrometer.

Results and discussion

Effect of iron on the γ-radiation-induced degradation of DNA bases

Quantification of a wide array of oxidative degradation products of DNA bases may be carried out by the gas chromatography–mass spectrometry assay in its standard version,[24] the reliability of which has been questioned. Indeed, artifactual oxidation in the derivatisation step[25,26] and flaws in the acidic hydrolysis of brittle lesions[15,27] have been observed. Therefore, quantification of the level of DNA bases damage was presently carried out by the highly specific and sensitive liquid chromatography coupled to electrospray ionisation-tandem mass spectrometry assay.[21] Several relevant radical-induced base lesions were quantified within isolated DNA exposed to γ-radiation under aerated conditions (Fig. 1 and 2). DNA was previously treated by a cation exchange resin to remove traces of transition metals. As previously shown,[21,28]

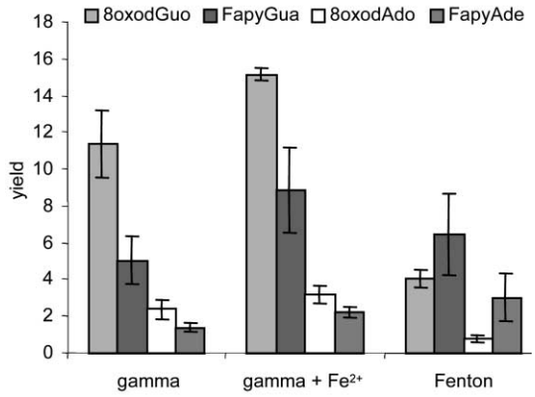


Fig. 1 Yield of formation of guanine and adenine degradation products in aerated aqueous solutions of isolated DNA exposed either to gamma radiation (in the absence or the presence of 100 μM Fe^{2+}–EDTA complex) or to the oxidising species produced upon Fenton reaction (in the presence of 100 μM Fe^{2+}–EDTA complex). Yields are expressed in lesions/10^6 bases per Gy and per μM H_2O_2, respectively.

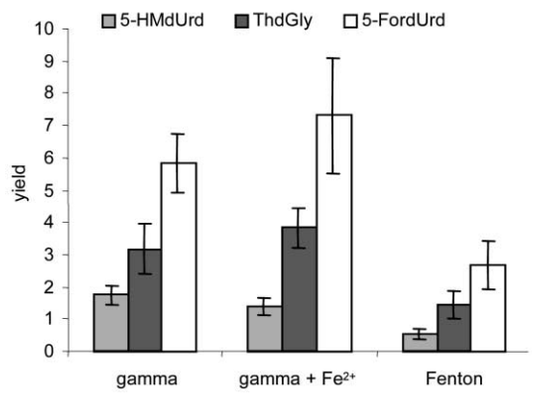


Fig. 2 Yield of formation of thymine oxidation products in aerated aqueous solutions of isolated DNA exposed either to gamma radiation (in the absence or the presence of 100 μM Fe^{2+}–EDTA complex) or to the oxidising species produced upon Fenton reaction (in the presence of 100 μM Fe^{2+}–EDTA complex). Yields are expressed in lesions/10^6 bases per Gy and per μM H_2O_2, respectively.

guanine, adenine and thymine were all targets for the $^{\bullet}OH$ radicals produced upon radiolysis of water. Addition of either Fe^{2+} or Fe^{3+} in the presence of EDTA to the irradiated solution did not change the relative yield of formation of the targeted modified DNA bases. This indicates that, for the quantified lesions, EDTA–ferrous ion complexes did not interfere with the radical base precursors produced upon γ-irradiation. Therefore, in the following comparison between DNA base damage produced by either γ-radiolysis or oxidation under Fenton reaction conditions, differences may be accounted for by the production of different reactive species.

DNA base damage produced under Fenton reaction conditions

The distribution of DNA damage produced upon treatment with transition metals has been extensively studied, mostly in terms of strand breaks and guanine oxidation products.[29–34] The formation of base damage induced by iron has been determined in both isolated[30–32,35] and cellular DNA.[36,37] However, the latter studies were based on GC-MS analyses. Their results have thus to be considered with caution for the reasons mentioned above. It may be pointed out that most of the studies involved the use of ferric ion while iron is active in its reduced ferrous ion. A reducing system has thus to be added in the reaction mixture. However, addition of a reducing species may alter the DNA base damage distribution and lead to false conclusions on the identity of the oxidising species that are implicated. Therefore, the present work was carried out with freshly prepared solutions of a Fe^{2+} salt. In addition, the availability of a sensitive technique for the quantification of base damage allowed to keep the amount of Fenton reagents below 200 μM. This is expected to minimise the occurrence of complex metal driven reactions. For a defined ferrous ion concentration, the level of damage was found to directly depend on the hydrogen peroxide concentration. The reported results represent the values inferred form the latter slopes (Fig. 1 and 2). The distribution of base lesions was similar to some extent to that generated upon γ-irradiation, as previously observed for damage to the 2-deoxyribose units.[38] However, some significant differences were observed. First, the ratio between the yield of FapyGua and that of 8-oxodGuo was three times higher than upon gamma-irradiation. A similar trend was observed for the related adenine degradation products. For both purine bases, the Fapy lesion and the 8-oxo derivative arise from the same 8-hydroxy-7,8-dihydropurine radical. The latter transient is then either oxidised into 8-oxopurine or reduced into the Fapy derivative.[10,39] Therefore, the observation of a significant increase in the relative yield of Fapy with respect to 8-oxopurine in the case of the Fenton reaction is strongly suggestive of the implication of a reducing species in the DNA degradation. However, as mentioned above, addition of ferrous ions and EDTA to γ-irradiated DNA solutions did not modify the base damage distribution. Another possibility could be the formation of superoxide anion but this may also be ruled out since $O_2^{\cdot -}$ is produced in significant yield upon γ-irradiation through the reaction of solvated electron with oxygen. As a consequence, the only likely explanation for the higher yield of Fapy derivatives is the production of a reducing species from the Fenton reagents. The latter could be generated upon reaction of $^{\bullet}OH$ with EDTA.[34] However, this seems unlikely because irradiation of DNA in the presence of free EDTA does not modify the base damage distribution (data not shown). In addition, Fenton reaction involving either ferrous ion–EDTA complex or free ferrous ions gives rise to the same base damage distribution (data not shown). It might be concluded that Fenton reaction degrades DNA through the production of $^{\bullet}OH$. However, an additional, yet unidentified species, exhibiting reducing properties is also produced that modulates the base damage distribution by enhancing the formation of Fapy derivatives of both adenine and guanine.

Indirect 2-deoxyribose-mediated base damage to DNA

In addition to DNA bases, 2-deoxyribose units represent other major targets for hydroxyl radicals within DNA.[38] As the result of $^{\bullet}OH$-mediated hydrogen abstraction from the sugar moieties of DNA, single stand breaks and abasic sites are produced, together with low molecular weight reactive aldehydic compounds. The latter have been recently proposed to further react with DNA bases, yielding exocyclic adducts first observed following exposure to chemicals and breakdown products of lipid peroxidation. Indeed, ethenobases[40,41] could be produced within DNA by reaction of the exocyclic amino groups of adenine, cytosine and guanine with phosphoglycolaldehyde,[18] a degradation product of 2-deoxyribose arising from the initial abstraction of the H3′ atom. Similarly, the MDA-2′-deoxyguanosine adduct (M1dGuo)[42,43] could be produced by reaction with base propenals[17,19] that are generated by initial abstraction of the H4′ atom of a deoxyribose ring. Therefore, we quantified the level of these exocyclic adducts in both γ-irradiated and Fenton-treated DNA samples in order to determine the contribution of the 2-deoxyribose mediated base lesions to the overall oxidative damage to DNA. For this purpose, the HPLC–MS/MS assay developed for the quantification of oxidised nucleosides in enzymatically digested DNA was extended to the measurement of M1dGuo, 1,N^2-εdAdo, 3,N^4-εdCyd and 1,N^2-εdGuo, as reported by other groups.[22,23] The level of oxidised bases and exocyclic adducts was measured

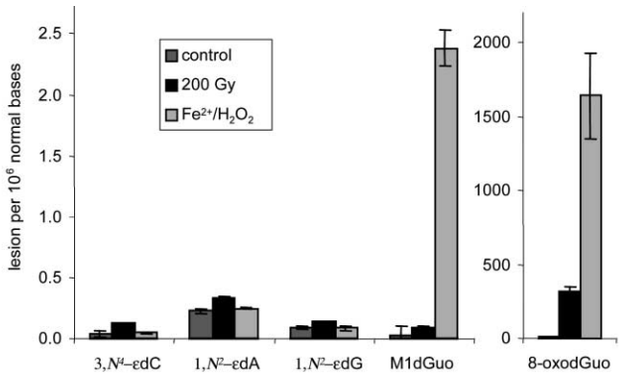


Fig. 3 Level of 1,N^2-εdAdo, 3,N^4-εdCyd, 1,N^2-εdGuo, M1dGuo and 8-oxodGuo (expressed in lesions per 10^6 normal bases) within isolated DNA either untreated, irradiated with 200 Gy of gamma radiation or exposed to Fenton reagent (Fe^{2+} 100 μM, H_2O_2 500 μM).

within DNA exposed either to gamma radiation or to Fenton reagents, in the presence and the absence of EDTA. As shown in Fig. 3, it was found that γ-irradiation failed to significantly increase the level of cyclic adducts while oxidised bases were produced in high yield. The formation of 1,N^2-εdAdo, 3,N^4-εdCyd and 1,N^2-εdGuo under the Fenton reaction conditions was also negligible. In contrast, a significant increase in the level of M1dGuo was observed with the latter oxidising system. However, the yield of M1dGuo was, at least, three orders of magnitude lower than that of the oxidised bases. Interestingly, when EDTA was added to the Fenton reaction mixture, a significant decrease in the yield of M1dGuo was observed, much more pronounced than that of 8-oxodGuo. Fig. 4

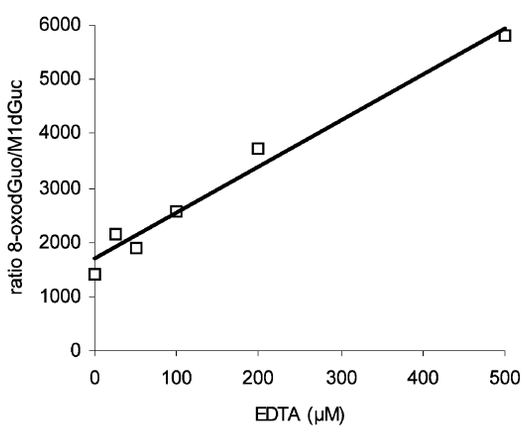


Fig. 4 Effect of the addition of EDTA on the ratio between the yields of formation of 8-oxodGuo and M1dGuo within isolated DNA exposed to Fenton reagents (Fe^{2+} 100 μM, H_2O_2 500 μM).

shows the increase in the value of the ratio between the yield of 8-oxodGuo and that of M1dGuo upon addition of increasing concentrations of EDTA. This observation suggests that free ferrous ions, able to bind to the 2-deoxyribose units of DNA, are required to yield the precursors of M1dGuo. This may be related to recent results[44] showing that, in contrast to γ-radiolysis, bleomycin, an iron based DNA binding antitumor agent, gives rise to base propenals, a proposed precursor of M1dGuo.[17,19]

Conclusion

By optimising reaction conditions and carrying out suitable control experiments, we were able to accurately compare the distribution of a wide set of base damage upon exposure to either γ-radiation or Fenton reagents. A first result is the minor influence of ferrous complexes on the radiation-induced degradation of DNA bases. This indicates that contamination of samples by traces of transition metal ions is unlikely to affect conclusions inferred from studies of the effect of ionizing radiation on DNA. Second, a similar pattern of oxidised bases was obtained under the two oxidising conditions. This allows in particular to rule out the contribution of one-electron oxidation processes in the Fenton reaction-induced DNA damage. Indeed, high degradation yield of guanine would have been observed because of the transfer of positive holes toward this base exhibiting the lowest oxidation potential within DNA.[45,46] However, a significant increase in the formation of the Fapy at the expense of the 8-oxo purine derivatives was observed upon treatment of DNA by Fe^{2+}–H_2O_2 with respect to γ-rays. This strongly suggests that reducing species are produced during the Fenton reaction. However, the change in the base damage profile is not specific enough under the latter conditions to allow its use as a marker of the Fenton reaction. 2-Hydroxyadenine has been suggested to be a specific product of the Fenton chemistry.[47] However, it appeared to be produced in very low yield within isolated DNA as inferred from HPLC–MS/MS measurements.[48] Therefore, other types of lesions were investigated, namely exocyclic adducts arising from the reaction of the oxidative degradation products of 2-deoxyribose with amino substituted DNA bases. None of the studied compounds was significantly produced by $^{\bullet}OH$ while the malondialdehyde-2′-deoxyguanosine adduct was generated, though in low yield, under the Fenton reaction conditions. It might thus be used as a marker of iron-induced oxidative damage to DNA. However, this will be limited to naked DNA since the yield of formation of M1dGuo is three orders of magnitude lower than that of 8-oxodGuo.

Abbreviations

1,N^2-εdAdo: 1,N^2-Etheno-2′-deoxyadenosine; 1,N^2-εdGuo: 1,N^2-etheno-2′-deoxyguanosine; 3,N^4-εdCyd: 3,N^4-etheno-2′-deoxycytidine; 5-FordUrd: 5-formyl-2′-deoxyuridine; 5-HMdUrd: 5-(hydroxymethyl)-2′-deoxyuridine; 8-oxodAdo: 8-oxo-7,8-dihydro-2′-deoxyadenosine; 8-oxodGuo: 8-oxo-7,8-dihydro-2′-deoxyguanosine; EDTA: Ethylenediaminetetraacetic acid; FapyAde: 4,6-diamino-5-formamidopyrimidine; FapyGua: 2,6-diamino-4-hydroxy-5-formamidopyrimidine; HPLC-MS/MS: high performance liquid chromatography coupled to tandem mass spectrometry; M1dGuo: pyrimidino-purine malonaldehyde-2′-deoxyguanosine adduct; ThdGly: *cis* and *trans* diastereoisomers of 5,6-dihydroxy-5,6-dihydrothymidine (thymidine glycols).

References

1 K. B. Beckman and B. N. Ames, *J. Biol. Chem.*, 1997, **272**, 19633–19636.
2 S. I. Liovech and I. Fridovich, *Free Radical Biol. Med.*, 1994, **16**, 29–33.
3 J. M. De Freitas and R. Meneghini, *Mutat. Res.*, 2001, **475**, 153–159.
4 C. Pourzand, R. D. Watkin, J. E. Brown and R. M. Tyrrell, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 6751–6756.
5 S. Toyokuni, *Free Radical Biol. Med.*, 1996, **20**, 553–566.
6 A. Barbouti, P.-T. Doullias, B.-Z. Zhu, B. Frei and D. Galaris, *Free Radical Biol. Med.*, 2001, **31**, 490–498.
7 H. C. Sutton and C. C. Winterbourn, *Free Radical Biol. Med.*, 1989, **6**, 53–60.
8 S. Goldstein, D. Meyerstein and G. Czapski, *Free Radical Biol. Med.*, 1993, **15**, 435–445.
9 D. A. Wink, C. B. Wink, R. W. Nims and P. C. Ford, *Environ. Health Perspect.*, 1994, **102**(suppl 3), 11–15.
10 S. Steenken, *Chem. Rev.*, 1989, **89**, 503–520.
11 J. Cadet, M. Berger, T. Douki and J.-L. Ravanat, *Rev. Physiol. Biochem. Pharmacol.*, 1997, **131**, 1–87.
12 Y. Luo, E. S. Henle and S. Linn, *J. Biol. Chem.*, 1996, **271**, 21167–21176.
13 E. S. Henle, Y. Luo, W. Gassmann and S. Linn, *J. Biol. Chem.*, 1996, **271**, 21177–21186.

14 M. Berger and J. Cadet, *Z. Naturforsch. B: Biosci.*, 1985, **40**, 1519–1531.
15 T. Douki, R. Martini, J.-L. Ravanat, R. J. Turesky and J. Cadet, *Carcinogenesis*, 1997, **18**, 2385–2391.
16 T. Douki, S. Spinelli, J.-L. Ravanat and J. Cadet, *J. Chem. Soc., Perkin Trans. 2*, 1999, 1875–1880.
17 P. C. Dedon, J. P. Plastaras, C. A. Rouzer and L. J. Marnett, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 11113–11116.
18 W. R. Jones and P. C. Dedon, *J. Am. Chem. Soc.*, 1999, **121**, 9231–9232.
19 J. P. Plastaras, J. N. Riggins, M. Ottender and L. J. Marnett, *Chem. Res. Toxicol.*, 2000, **13**, 1235–1242.
20 C. Chapeau and L. J. Marnett, *Chem. Res. Toxicol.*, 1991, **4**, 636–638.
21 S. Frelon, T. Douki, J.-L. Ravanat, C. Tornabene and J. Cadet, *Chem. Res. Toxicol.*, 2000, **13**, 1002–1010.
22 D. R. Doerge, M. I. Churchwell, J.-L. Fang and F. A. Beland, *Chem. Res. Toxicol.*, 2000, **13**, 1259–1264.
23 D. W. Roberts, M. I. Churchwell, F. A. Beland, J.-L. Fang and D. R. Doerge, *Anal. Chem.*, 2001, **73**, 303–309.
24 M. Dizdaroglu and E. Gajewski, *Methods Enzymol.*, 1990, **186**, 530–544.
25 J.-L. Ravanat, R. J. Turesky, E. Gremaud, L. J. Trudel and R. H. Stadler, *Chem. Res. Toxicol.*, 1995, **8**, 1039–1045.
26 T. Douki, T. Delatour, F. Bianchini and J. Cadet, *Carcinogenesis*, 1996, **17**, 347–352.
27 S. G. Swarts, G. S. Smith, L. Miao and K. T. Wheeler, *Radiat. Environ. Biophys.*, 1996, **35**, 41–53.
28 T. Douki, Y. Bretonnière and J. Cadet, *Radiat. Res.*, 2000, **153**, 29–35.
29 H. Kasai and S. Nishimura, *Nucleic Acids Res.*, 1984, **12**, 2137–2145.
30 J. A. Imlay, S. M. Chin and S. Linn, *Science*, 1988, **240**, 640–642.
31 M. L. Muiras, P. U. Giacomoni and P. Tachon, *Mutat. Res.*, 1993, **295**, 47–54.
32 J. W. Park and R. A. Floyd, *Arch. Biochem. Biophys.*, 1994, **312**, 285–291.
33 L. J. Kennedy, K. Moore, J. L. Caulfield, S. R. Tannenbaum and P. C. Dedon, *Chem. Res. Toxicol.*, 1997, **10**, 386–392.
34 H. B. Ambroz, T. K. Bradshaw, T. J. Kemp, E. M. Kornacka and G. K. Przybytniak, *J. Photochem. Phototbiol. A: Chem.*, 2001, **142**, 9–18.
35 O. I. Aruoma, B. Halliwell and M. Dizdaroglu, *J. Biol. Chem.*, 1989, **264**, 13024–13028.
36 V. Abalea, J. Cillard, M. P. Dubos, J. P. Anger, P. Cillard and I. Morel, *Carcinogenesis*, 1998, **19**, 1053–1059.
37 T. Nunoshiba, F. Obata, A. C. Boss, S. Oikawa, T. Mori, S. Kawanishi and K. Yamamoto, *J. Biol. Chem.*, 1999, **274**, 34832–34837.
38 W. K. Pogozelski and T. D. Tullius, *Chem. Rev.*, 1998, **98**, 1089–1107.
39 L. P. Candeias and S. Steenken, *Chem. Eur. J.*, 2000, **6**, 475–484.
40 R. S. Sodum and F.-L. Chung, *Cancer Res.*, 1988, **48**, 320–323.
41 F. El Ghissassi, A. Barbin, J. Nair and H. Bartsch, *Chem. Res. Toxicol.*, 1995, **8**, 278–283.
42 H. Seto, K. Akiyama, T. Okuda, T. Hashimoto, T. Takesue and T. Ikemura, *Chem. Lett.*, 1981, 707–708.
43 A. K. Basu, S. M. O'Hara, P. Valladier, K. Stone, O. Mols and L. J. Marnett, *Chem. Res. Toxicol.*, 1988, **1**, 53–59.
44 R. Rashid, D. Langfinger, R. Wagner, H.-P. Schuchman and C. von Sonntag, *Int. J. Radiat. Biol.*, 1999, **75**, 101–109.
45 P. T. Henderson, D. Jones, G. Hampikian, Y. Kan and G. B. Schuster, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 8353–5358.
46 B. Giese, *Acc. Chem. Res.*, 2000, **33**, 631–636.
47 H. Kamiya and H. Kasai, *J. Biol. Chem.*, 1995, **270**, 19446–19450.
48 S. Frelon, T. Douki and J. Cadet, *Free Radical Res.*, 2002, **36**, 499–508.