7,8-Dihydro-8-oxo-2′-deoxyguanosine Residues in DNA Are Radiation Damage "Hot" Spots in the Direct γ Radiation Damage Pathway

Zara A. Doddridge, Paul M. Cullis,*,† George D. D. Jones,* and Mark E Malone‡

Department of Chemistry and Center for Mechanisms of Human Toxicity University of Leicester, Leicester, LE1 7RH, UK

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7,8-Dihydro-8-oxo-guanine (8-oxo-G) is a major DNA base modification associated with damage caused by ionizing radiation, reactive oxygen species (particularly hydroxyl radicals),[1] chemical oxidation,[2] and photoionization.[3] The 8-oxo-G lesion is thought to be involved in mutagenesis, carcinogenesis, and aging.[4,5] Furthermore, it is apparent that significant steady-state levels of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG) residues exist in genomic DNA.[6] In a recent biphotonic photoionization study, we reported unambiguous evidence that the guanine radical cation of 2′-deoxyguanosine is a precursor of 8-oxo-dG.[7] We also demonstrated that photoionization of oligodeoxynucleotides containing an 8-oxo-dG residue leads to the site-specific formation of an alkali-labile site at this residue and speculated that 8-oxo-dG residues might represent radiation-sensitive "hot" spots in DNA.[7] We report here the first evidence for sequence-specific damage leading to alkali-labile sites in oligodeoxynucleotides containing 8-oxo-dG when γ-irradiated under condition of direct damage. This implies that efficient charge migration competes with events leading to "trapping" of the initial radical damage even at ambient temperatures.

Ionizing radiation-induced damage to DNA occurs via two limiting mechanisms. The most widely studied of these, which operates in dilute aqueous solution, is the indirect pathway where the initial energy deposition occurs in the solvent and subsequent damage to DNA occurs through the radiolysis products of water, particularly the hydroxyl radical.[8] In contrast to this, the direct pathway involves direct ionization of DNA leading to electron loss and electron gain centers, which may be of importance in genomic DNA. The principal electron loss center is widely accepted to be the guanine base leading to the guanine radical cation which has been characterized at low temperature by ESR spectroscopy.[9] The identification of the major electron gain center has been more controversial, although it is now generally agreed that it is a population of the two pyrimidine radical anions, with initial ratios of $C^{\bullet-}$ to $T^{\bullet-}$ being approximately 4 to 1.[10] We and others have shown that, under conditions of direct irradiation, charge migration can occur over quite large distances within DNA.[11] Indeed the product distribution of oxidative damage in DNA has been studied as a function of the degree of hydration, and 8-oxo-dG has been identified as a major product consistent with charge migration.[12] The efficacy of this charge migration has important implications in terms of the potential to transfer damage to sites remote from where the initial ionization event took place.

Irradiation of a 5′-^{32}P-labeled oligo-dA 21mer with 8-oxo-dG (**X**) at the central position (oligo-**1**; 5′-^{32}P-d(AAAAAAAAAA**X**-AAAAAAAAAA)) as a "dry" film led to no immediate strand breaks detectable by polyacrylamide gel electrophoresis (PAGE), Figure 1a.[13] However, treatment of the γ-irradiated oligodeoxynucleotide sample with aqueous piperidine led to one major band on PAGE analysis (Figure 1b) that comigrated with a 10mer with a phosphate end group (from the Maxam−Gilbert sequencing lane) implying cleavage of the phosphodiester 5′ to the 8-oxo-dG site. The unirradiated oligodeoxynucleotide when treated with aqueous piperidine gave only a small amount of strand cleavage at the 8-oxo-dG residue (Figure 1b, lane 1), consistent with our previous observations.[7] Analogous experiments in a sequence that is more DNA-like (oligo-**2**; 5′-^{32}P-ATGCATGCAT**X**CATGCATGC-3′) gave the same results, with radiation-dependent piperidine cleavage occurring selectively at the 8-oxo-dG site in a dose-dependent manner, Table 1. Accurate quantitation of the extent of radiation-dependent piperidine-induced cleavage is complicated by the slow piperidine cleavage of intact 8-oxo-dG residues themselves. Nevertheless, the trend in the dose dependence is clear in both the Figure and the data in Table 1.

We have previously shown that γ-irradiation of DNA under conditions of direct damage leads to non-sequence-specific cleavage, giving rise to predominantly phosphate end groups.[14] In light of recent work by Steenken et al.,[15] this nonspecific cleavage may have arisen from direct ionization of the sugar phosphate backbone. The estimated yields for this direct strand cleavage at an individual site are extremely low[8,14] (*G* value in μmol J^{-1} ca. 3×10^{-6}) and hence would represent an extremely low background cleavage in this study. The observation that γ-irradiation of an 8-oxo-dG-containing oligodeoxynucleotide leads to sequence specific latent strand breaks is the first clear example of sequence specificity in processes involving high energy γ-irradiation. By analogy with the photoionization study, damage to the 8-oxo-dG presumably occurs through the oxidative branch of the direct mechanism. The initial electron ejection must occur from a number of sites, but the observations of selective damage to the 8-oxo-dG site provides evidence for efficient hole-transfer to the 8-oxo-dG site as might be expected since this clearly has the lowest ionization potential.[16] This observation is analogous to that seen in the photoionization of unmodified DNA

† E-mail: PMC@leicester.ac.uk.
‡ Present address: Unilever UK Ltd, Colworth House, Bedford, UK.

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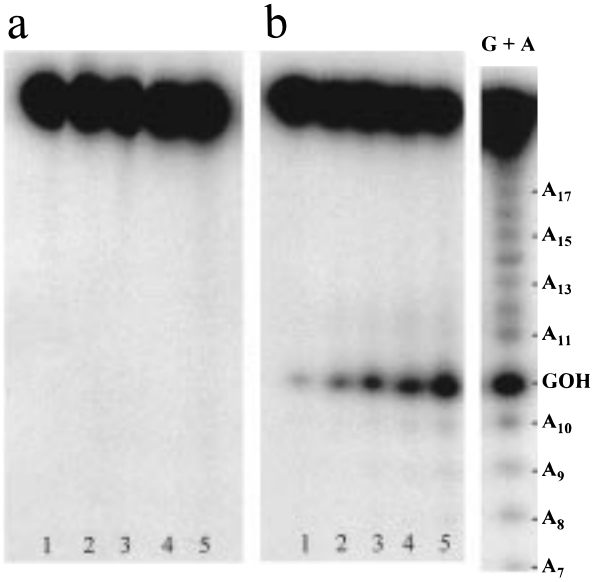


Figure 1. Phosphorimager gel scan of the products of the γ irradiation of a dry film oligo-**1** (5′-^{32}P-d(AAAAAAAAAA**X**AAAAAAAAAA)) showing a dose-dependent generation of a piperidine sensitive lesion. **1a**: lanes 1−5 are samples of the oligo-**1** irradiate for 0, 6.5, 24, 30.5, and 48 h respectively; **1b**: lanes 1−5 are the same samples as in 1a following piperidine cleavage (0.1 M, 90 °C for 1 h); **1c** Maxam−Gilbert G and A sequencing lane.

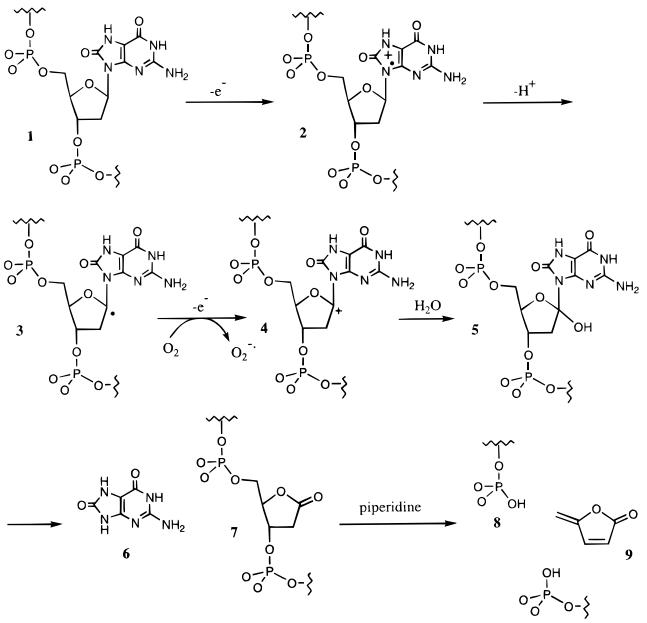
Table 1.

irradiation time[a] (h)	% cleavage at 8-oxo-dG oligo-**1**[b,c]	% cleavage at 8-oxo-dG oligo-**2**[b,c]
6.5	7	8
24	10	n.d.
30.5	15	11
48	24	16

[a] The dose rate of the ^{60}Co source was 1.039 kGy/hour. [b] 2.5 pmoles of oligonucleotide were irradiated as thin films. [c] The data have been corrected for the slow piperidine cleavage at intact 8-oxo-dG sites, and the values represent the excess % piperidine-induced cleavage compared to the unirradiated controls.

where hole-transfer from adenine, thymine, and cytidine bases to the guanine base leads to an excess of G-specific damage.[7,17] Obviously, this study has focused on sites that become piperidine sensitive on γ-irradiation and this might be biased in favor of the product from 8-oxo-dG. However, our previous photoionization studies on DNA demonstrated that there is some degree of piperidine-dependent cleavage at all sites in competition with hole-transfer to G.[7] As we observed with the photoionization studies, when the "hole trap" is even deeper, as is the case with the 8-oxo-dG-containing oligodeoxynucleotides, hole-transfer is even more efficient. This conclusion is further supported by estimates of the yields for the modification at the 8-oxo-dG. The "apparent" *G* value for the modification of the 8-oxo-dG site is ca. 1.3 if only $^1/_{21}$ of the energy deposited in the oligonucleotide is considered. If complete hole migration is assumed, the estimated *G* value is 0.059 which is close to the literature *G* value for *total base release* on irradiation of solid DNA under oxic conditions.[18] It is also necessary to comment on the products of the reduction pathway. Many groups have noted that γ-irradiation of solid DNA also leads to the radical anions of the pyrimidine bases, which is in contrast to what is observed by ESR

Scheme 1



spectroscopy in the photoionization studies.[19] With respect to this present study, either the electron is not efficiently trapped by the oligonucleotide, or the products from the radical anion do not give rise to alkali-labile products.

As yet we have no definitive evidence concerning the mechanism of the reaction or the nature of the alkali-labile site. However, we have previously reported that photoionization of nucleoside 8-oxo-dG led to release of 8-oxo-G which was suggestive of a C-1′ radical intermediate. Assuming that the γ radiolysis experiment is also going through the oxidative manifold then the most reasonable candidate for the alkali-labile site is the deoxyribolactone lesion, Scheme 1. The observation of very low levels of immediate strand cleavage, with the majority of the lesion being piperidine sensitive is consistent with the mechanism shown in Scheme 1 where the lactone (**7**) is formed in the intact chain. In contrast to the permanganate oxidation studies reported by Koizume et al.,[20] we have not observed significant levels of piperidine-induced cleavage at sites flanking the 8-oxo-dG either in the photoionization studies[7] or in these current ionizing radiation experiments. The precise mechanism of the modification of sites remote from the 8-oxo-dG in the permanganate oxidation studies is unclear.

In γ-irradiation studies on DNA, although both hole migration to give mainly $G^{\bullet+}$ and electron transfer to give $C^{\bullet-}$ and/or $T^{\bullet-}$ have been observed by ESR spectroscopy[10,11a,b] at low temperature, hitherto it has not been possible to demonstrate a similar specificity in subsequent chemical damage. Clearly, the possibility of relatively long-range charge migration in DNA means that the radiochemistry of modified DNA bases that exist or can be formed within cellular DNA may be crucially important, particularly in terms of potential radiation hot spots. This present study has demonstrated that, in line with our earlier speculation, 8-oxo-dG can indeed function as one of these.

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