Novel DNA damage mediated by oxidation of an 8-oxoguanine residue

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Permanganate oxidation of 7,8-dihydro-8-oxoguanine in DNA oligomers causes damage to the neighbouring nucleotide residues; a highly reactive residue is the guanine next to the 8-oxoguanine.

7,8-Dihydro-8-oxoguanine (8-oxo-G, often called 8-hydroxyguanine) is a modified base generated in DNA by a variety of reagents, ionizing radiation and endogenous oxidation processes that produce reactive oxygen species,[1] and by photooxidation.[2] The DNA lesion is known to be involved in mutagenesis, carcinogenesis and ageing.[1,3] Very little is known about the chemical behaviour of 8-oxo-G in DNA, although the biological properties[4] and base pairing abilities[5,6] of the modified base in DNA and the related repair enzymes[7] are widely studied. For example, 7,8-dihydro-8-oxoguanosine has unique redox properties;[8] however, there is no precedent for the oxidation of 8-oxo-G residues in DNA. If the event is possible *in vivo*, the mutagenic potency of the oxidation product(s) of 8-oxo-G should be considered. We report here a novel potassium permanganate reaction of oligodeoxyribonucleotides containing an 8-oxo-G residue: The reaction that is initiated by the oxidative modification of 8-oxo-G causes damage to the neighbouring nucleotide residues.

The 12-mer oligonucleotides containing 8-oxo-G (**1**–**7**) used in this study are shown in Table 1 and were synthesized as described previously.[6] Oligonucleotides **1** and **2** correspond to a part of the human c-Ha-*ras* gene that contains a modified codon 12, and the others have altered base compositions. Oligonucleotides **1**–**7** were labelled at the 5′-ends with ^{32}P and were treated with 120 μmol dm^{-3} potassium permanganate in 10 mmol dm^{-3} sodium phosphate buffer (pH 7.0)† for 15 min. at 25 °C. In order to examine the damage at the 8-oxo-G position, the DNAs were subjected to hot alkaline treatment with 1 mol dm^{-3} piperidine for 30 min. at 90 °C, followed by denaturing 20% polyacrylamide gel electrophoresis (PAGE). In the case of substrate **1**, strand scission was found to occur efficiently at the 8-oxo-G position,‡ but unexpectedly, a small amount of scission was also observed at the 5′-upstream G position, three bases distant from the modified base (Fig. 1, lane 5, and Table 1). Since the cleavage sites could be identified from the comigrated Maxam–Gilbert sequencing ladders, and no cleavage was observed without piperidine treatment (Fig. 1, lane 6), the 5′-end-labelled cleavage products seemed to be produced *via* an abasic site intermediate. Also, unexpected but efficient scission was observed at the G site 5′-adjacent to the 8-oxo-G (Fig. 1, lane 9) when the permanganate reaction was carried out using strand **2**.§ The scission yields at this and the 8-oxo-G positions were 53 and 36%, respectively. Namely, about 90% of **2** (precisely, the 5′-side region with 8-oxo-G) was damaged by the permanganate reaction, whereas the nascent oligonucleotides with G instead of 8-oxo-G for **1** and **2** were not cleaved at any position, except for slight damage of the thymine at the second position from the 3′-end (Fig. 1, lane 13).¶ The reaction of strand **3**, with the sequence -G-C-G-G-(8-oxo-G)-, produced damage to the two continuous Gs and the 5′-upstream G (Table 1).

To investigate the sequence- and base-specificity of this reaction, we used other strands. Strands **4** and **5** are analogous to **2**, but they contain A and T instead of G at the site adjacent to the 5′-side of the 8-oxo-G. The reaction of **4** and its workup led to scission at the 8-oxo-G position (69% yield) and the 5′-upstream G position (7.9% yield) four bases distant from the modified base; scission at the adenine position was not detected, as in the case of the control experiment (the damage of the adenine base will be discussed later). Strand **5** was cleaved at the 5′-flanking T and the 5′-upstream G with 27 and 4.3% yields, respectively, whereas the control strand gave a 7.3% yield of the T site scission and no scission of the G site. Strand

Table 1 Sequences, cleavage positions and percentages of 5′-end-labelled oligodeoxynucleotides[a]

Compound	Sequence	Cleavage yield (%)
1	5′GC**G**CC**G**GCGGTG3′	4.8 76
2	5′GCGCC**GG**CGGTG3′	53 36
3	5′GC**G**C**GGG**CGGTG3′	2.7 13 26 32
4	5′GC**G**CCA**G**CGGTG3′	7.9 69
5	5′GC**G**CCT**G**CGGTG3′	4.3 27 51
6	5′AT**G**AC**GG**AATAT3′	16 44 25
7	5′TT**TTCG**TTCCTT3′	28 27 6.6 33

[a] All reactions were performed under the conditions shown in Fig. 1. G and bold letters refer to 8-oxoguanine and cleavage sites. Cleavage percentages correspond to those for sites with 5′ to 3′ shown at the left.

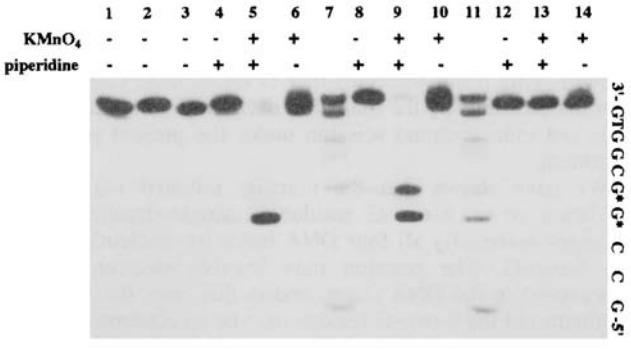

Fig. 1 Autoradiogram of reaction products from **1** and **2**, and the nascent strand after 20% polyacrylamide gel (denaturing 7 mol dm^{-3} urea) electrophoresis. The oligonucleotides **1** and **2**, and the nascent strand were labelled at the 5′-ends with ^{32}P. Solutions (20 μl) buffered to pH 7.0 (10 mmol dm^{-3} sodium phosphate), and containing 0.5 μmol dm^{-3} oligonucleotide and 120 μmol dm^{-3} permanganate, were maintained at 25 °C for 15 min. and were quenched with 5 μl of allyl alcohol. The samples were individually added to 25 μl of 2 mol dm^{-3} piperidine, maintained at 90 °C for 30 min., lyophilized, coevaporated with water (40 μl × 3), and dissolved in gel loading solution containing 5 mol dm^{-3} urea. The samples were heated to 55 °C and chilled quickly for electrophoresis. Lanes 1, 4, 5, 6: **1**; Lanes 2, 8, 9, 10: **2**; Lanes 3, 12, 13, 14: the nascent strand; Lanes 7, 11: Maxam-Gilbert A + G lanes for **1** and **2**, respectively. G* designates G or 8-oxoguanine.

6 is A,T-rich as compared with strands **1–5**, and the strand scission occurred at the 8-oxo-G, the 5′-flanking G, and the 5′-upstream G. The scission-site pattern was similar to that obtained for the reaction of **5**. The reaction of **7**, which has T-rich pyrimidine sequences on both sides of the 8-oxo-G, revealed cleavages at the two Ts upstream of the 8-oxo-G, as well as minor cleavage of the C flanking the 8-oxo-G on the 5′ side. Analysis of the reactions of strands **5** and **7** revealed that the strand scissions at the 5′-flanking T, or at the positions two (or three) bases distant, were greatly enhanced as compared with those of the nascent strands (data for **5** were indicated above). These results with the DNA oligomers strongly suggest that the reaction (oxidation) of the 8-oxo-G residue with $KMnO_4$ initiates damage of the neighbouring nucleotide residues, and sequence-specificity of the reaction is unlikely.

To examine the damage to the 3′-side sequence of the 8-oxo-G residue, oligonucleotides (**1,2,5,7**) were 3′-end-labelled with [α-^{32}P]ddATP plus terminal deoxynucleotidyl transferase, and were used for the above reaction. The results show that the G and T positions at the 3′-side were also modified efficiently, as well as those at the 5′-side. From the experiments with 5′- and 3′-end-labelled strands, it appears that as the position of the G (or T) moves further away from the 8-oxo-G, the scission at the G (or T) position becomes less efficient. Furthermore, it is suggested that the apparent high and low reactivities of the G and C sites, respectively, implicate a redox process. Among the four common bases, the G and C bases have the lowest and highest redox potentials, respectively.‖

To investigate the mode of the strand damage, a mixture of non-labelled strand **6** and the 5′-end-labelled nascent strand, with G instead of 8-oxo-G, was treated with permanganate and then with piperidine. The nascent strand was not cleaved at any of the three G positions, while the resulting scissions at the two T residues (positions 1 and 3 from the 3′-end) were unchanged, and were independent of the presence or absence of **6**. Thus, we could eliminate the possibility of inter- and intra-molecular reactions of the oxidation product(s) of the 8-oxo-G residue *via* diffusional contact with the nucleotide residues.

The modification of the adenine positions in the DNA reaction products was then investigated by enzymic digestion of the products from **4** with snake venom phosphodiesterase and alkaline phosphatase. HPLC analysis of the resulting nucleosides shows that the amount of 7,8-dihydro-8-oxo-2′-deoxyguanosine and the adjacent 2′-deoxyadenosine greatly decreased (about 100 and 70% respectively) after the permanganate treatment, although the 2′-deoxyadenosine was intact in the control experiments with the nascent strand of **4** (data not shown). This result indicates that DNA damage can occur at adenine positions by the oxidation of 8-oxo-G, but the damage does not induce strand scission under the present piperidine treatment.

We have shown that the reaction initiated *via* $KMnO_4$ oxidation of an 8-oxo-G residue in single-stranded DNAs damages essentially all four DNA bases (or nucleotides) near the 8-oxo-G. The reaction may involve electron transfer process(es) in the DNA chain, and in this case, the oxidation product(s) of the 8-oxo-G residue may be an electron acceptor. It has been proposed that photosensitized formation of 8-oxoguanine in DNA[2] and photoinduced DNA cleavage[9] involve the electron transfer from G residues to photoexcited molecules (the formation of a guanine radical cation[10]). Indeed, a very recent report on laser photolysis of single-stranded DNA oligomers indicates that migration of oxidative damage (radical cations of bases) occurs between certain neighbouring bases.[10c]

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Footnotes

† All reactions of the oligomers were performed in buffer lacking salts such as NaCl in order to avoid the formation of higher ordered structures, like a guanine tetraplex DNA (S. S. Smith, A. Laayoun, R. G. Lingeman, D. J. Baker, J. Riley, *J. Mol. Biol.*, 1994, **243**, 143).

‡ A DNA strand containing 8-oxo-G was cleaved slightly at the modified base position with hot piperidine treatment alone, as can be seen in Fig. 1, lanes 4 and 8 (M.-H. Chung, H. Kiyosawa, E. Ohtsuka, S. Nishimura and H. Kasai, *Biochem. Biophys. Res. Commun.*, 1992, **188**, 1).

§ With the present reaction conditions, the strand scission was observed exclusively at the 8-oxo-G position within 30 s of the reaction initiation.

¶ It is known that permanganate oxidation of thymines in single-stranded DNA and following piperidine treatment effectively cleaves the strand at the thymine positions, although those in duplex DNA are resistant (H. Hayatsu and T. Ukita, *Biochem. Biophys. Res. Commun.*, 1967, **29**, 556; J. G. McCarthy, *Nucleic Acids Res.*, 1989, **17**, 7541).

‖ Redox potentials of DNA bases are as follows: guanine, +1.29; adenine, +1.39; thymine, +1.49; cytosine, +1.64 (in V *vs.* saturated calomel electrode) (L. Kittler, G. Löber, F. A. Gollmick and H. Berg, *Bioelectrochem. Bioenerg.*, 1980, **7**, 503).

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