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DNA STRAND CLEAVAGE AT 8-HYDROXYGUANINE RESIDUES BY HOT PIPERIDINE TREATMENT*

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SUMMARY: The two findings indicate that 8-hydroxyguanine(8-OH-Gua) is a hot piperidine-sensitive lesion in DNA. These are cleavages of DNA containing 8-OH-Gua at the site of this residue and decomposition of 8-hydroxydeoxyguanosine when the DNA and nucleoside were treated in 1 M piperidine for 30 min at 90°C. However, no cleavage was observed in DNA containing 8-hydoxyadenine or O^6-methylguanine. 8-OH-Gua was found to be different from apurinic sites that are also alkali-labile lesions since the former was more resistant to alkali treatment. This property of 8-OH-Gua can be used as a check for the incorporation of this base into DNA after the synthesis of DNA containing 8-OH-Gua at a specific position or possibly can be one of the markers for the identification of 8-OH-Gua formed in DNA exposed to reactive oxygen species.

In DNA treated with Cu(II) and H_2O_2, Sagripanti and Kraemer(1) observed a guanine lesion where the DNA was easily cleaved by heating in 1 M piperidine for 30 min at 90°C. In the same study, they also showed that the hot piperidine-sensitive guanine lesion did not block *in vitro* DNA polymerization. Apart from learning that the guanine lesion was at least not due to the loss of guanine residues, the nature of the lesion has not been explored yet.

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Abbreviations : 8-OH-Gua; 8-hydroxyguanine, 8-OH-Ade; 8-hydroxyadenine, 8-OH-dG; 8-hydroxydeoxyguanosine, O^6-MeGua; O^6-methylguanine, AP; apurinic.

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On the other hand, among several types of lesions identified in DNA exposed to reactive oxygen species, there has been reported one modification well defined on guanine residues, i.e. 8-hydroxyguanine(8-OH-Gua). The 8-OH-Gua can be formed very easily *in vivo* (2, 3) as well as *in vitro* (4) by various agents producing reactive oxygen species. This modified residue also permits the DNA polymerization to bypass(5).

The findings described above allowed us to make a simple assumption that 8-OH-Gua may be labile to hot piperidine like the guanine lesion produced in Cu(II)-H_2O_2-treated DNA. In the present study, we tested the assumption using a synthetic DNA that contains 8-OH-Gua at a defined position and demonstrated that the 8-OH-Gua residue in DNA was a spot where the DNA strand was easily scissioned by hot piperidine treatment.

MATERIALS and METHODS

Preparation of Oligodeoxynucleotides

The oligodeoxynucleotides containing 8-OH-Gua, 8-hydroxyadenine(8-OH-Ade) or O^6-methyl guanine(O^6-MeGua) were synthesized as previously described(6). Other oligodeoxynucleotides were synthesized on an Applied Biosystem and purified according to the manufacturer's procedures. The apurinic(AP) DNA was prepared from an oligo dT containing one residue of dA by incubation in 100 µl of 30 mM HCl and 1 mM EDTA at 37°C for 24 h(7) and purified on a 20% polyacrylamide gel by elution from gel slices. Fig. 1 shows the sequences of these oligodeoxynucleotides. The oligodeoxynucleotides were terminally labeled at the 5'-end using [γ-^{32}P] ATP(Amersham; specific activity, 5000 Ci/mmole) and T4 -polynucleotide kinase(Takara Shuzo Co., Japan)(6) or at the 3'-end using [α-^{32}P] cordycepin(NEW Research Products, du Pont) and deoxynucleotidyl-terminal transferase(Takara Shuzo Co., Japan)(8)

Detection of Strand Scission by Hot Piperidine

Each of the labeled DNAs (1 pmol) was heated for 30 min in 100 µl of 1 M piperidine at 90°C and reaction products were analyzed by a denaturing polyacrylamide gel (20%) electrophoresis according to the procedure of Maxam and Gilbert (9).

HPLC Analysis of Hot Piperidine-Treated 8-Hydroxydeoxyguanosine

8-Hydroxydeoxygnanosine(8-OH-dG) was also treated with 1 M piperidine as described above and the reaction was analyzed with a high pressure liquid chromatography(HPLC) equipped with a photodiode array UV detector(Hewlett Packard).

RESULTS

To test whether 8-OH-Gua is a hot piperidine lesion or not, the end-labeled 8-OH-Gua-containing 46 mer DNA(Fig. 1, No. 2) and 46 mer DNA with normal G in place of 8-OH-Gua(Fig. 1, No. 1) were treated with 1 M piperidine

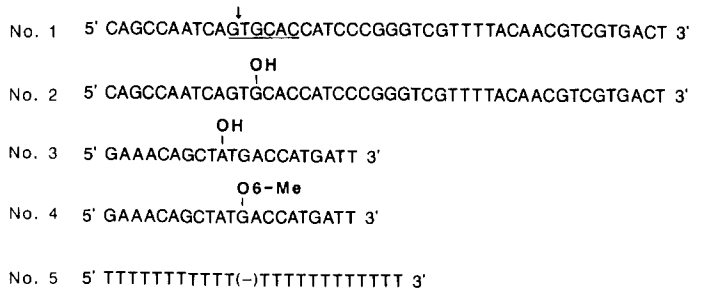


Fig. 1. Sequences of DNAs used in the present study. No.1; unmodified 46 mer DNA which has an ApaL1 restriction site to test the presence of 8-OH-Gua, No. 2; the same as No. 1 but one G in ApaL1 site is replaced with 8-OH-Gua. No. 1 was cut by ApaL1 but No. 2 was not. No. 3, 4 and 5; oligodeoxynucleotides containing 8-OH-Ade, O^6-MeGua and AP site, respectively.

at 90°C for 30 min, and analyzed by electrophoresis in a sequencing gel(Fig. 2). The unmodified 46 mer DNA was sequenced in parallel on the same gel to determine the position of scission and the nucleotides invloved. Whether labeled at the 5'-end(Fig. 2A) or the 3'-end(Fig. 2B), only the DNA containing 8-OH-Gua was cleaved to give a product running at the position of band G in the sequence ladder corresponding to the position of 8-OH-Gua(Fig. 2A, lane A2 and Fig. 2B, lane PPRD). The cleavage was also observed on the 8-OH-Gua-containing duplex DNA(Fig. 2, lane B2). But the unmodified DNA failed to give any cleavage products(Fig. 2A, lanes C2 and D2).

In addition to the strand scission of DNA containing 8-OH-Gua, the damage to this modified base by hot piperidine was also assessed at nucleoside level. When 8-OH-dG was treated with 1 M piperidine at 90°C for 30 min and analyzed by HPLC, the UV peak of 8-OH-dG was decreased(cf. peak 1 in curves a and b of Fig. 3A) and at the same time a new peak appeared(peak 2 in curve b of Fig. 3A). In the control experiment the peak 3 in curve b of Fig. 3A was confirmed to be an unknown material present in piperidine solution. In Fig. 3B, it was observed that the spectrum of the new product(dotted line) differed from that of the peak of 8-OH-dG(dashed line). These results clearly indicate that 8-OH-dG is decomposed by the hot piperidine treatment. But the nature of the decomposition product was not explored further.

In contrast to 8-OH-Gua, 8-OH-Ade and O^6-MeGua were resistant to the hot piperidine treatment. As shown in Fig. 4, no cleavage product was observed from the DNAs containing 8-OH-Ade or O^6-MeGua treated with hot piperidine(lanes 3 and 4) (the autoradiograph of Fig. 4 shows a high background because of the longer exposure of the film to the polyacrylamide gel in order to detect any cleavage products).

The alkali labile property of 8-OH-Gua was compared to that of AP sites. As a positive control, an AP site-containing DNA (No. 5 in Fig. 1) was prepared. The

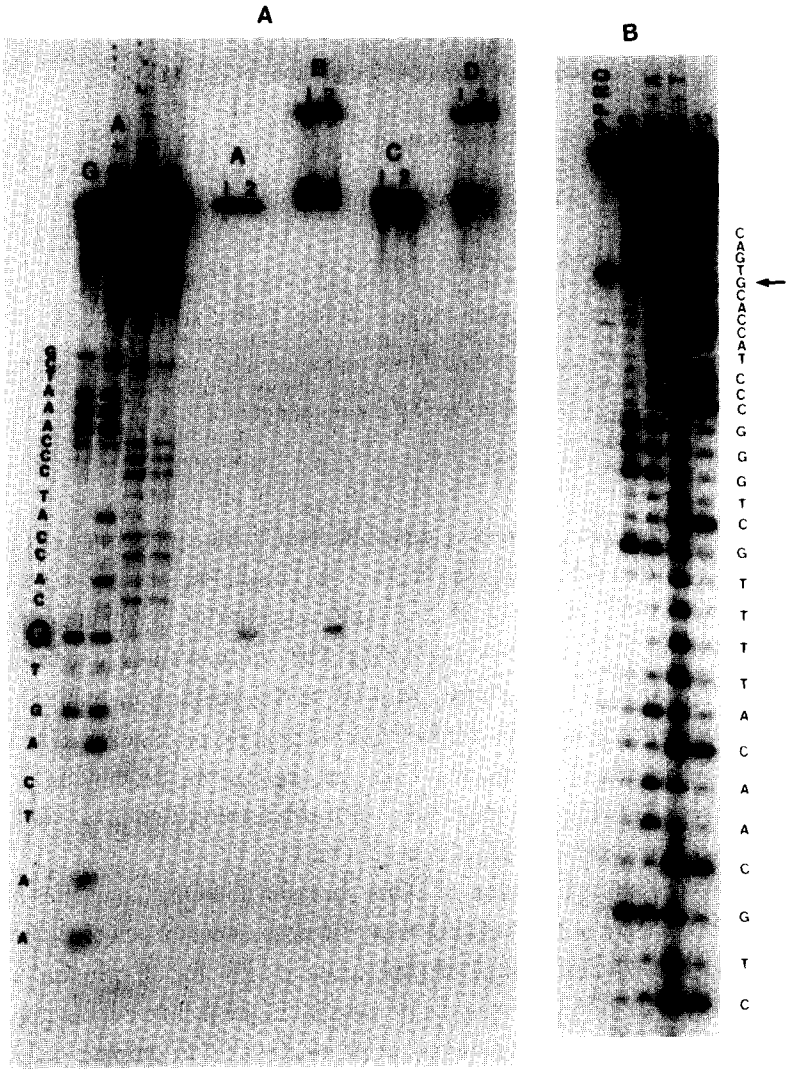
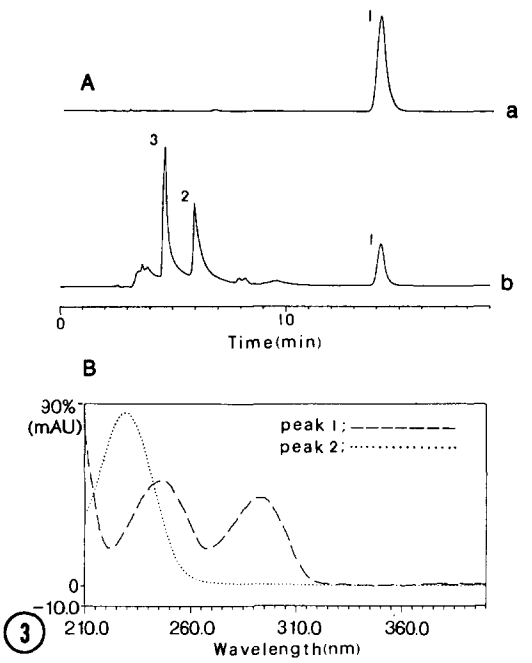


Fig. 2. Cleavage of 8-OH-Gua-containing DNA by hot piperidine treatment. Panel A. 5'-end labeled 46 mer DNA containing 8-OH-Gua or normal G replacing 8-OH-Gua was treated with 1 M piperidine at 90°C for 30 min. When loaded into the gel, DNA samples were dissolved in a loading buffer(80% formamide, 10 mM NaOH, 0.1% xylene cyanol and 0.1% Bromophenol Blue) and heated for 3 min at 90°C. Lanes A and B; single stranded(ss) and double stranded(ds) 8-OH-Gua containing DNA, respectively,and lanes C and D; ss and ds unmodified DNA, respectively. Duplex DNAs were obtained by mixing complementary strands at the ratio of 1:1, heating to 70°C for 10 min and cooling to room temperature. In each paired lane, lanes 1 and 2 are without and with piperidine treatment, respectively. The four lanes on the left are Maxam-Gilbert reactions of unmodified 46 mer DNA. Bold-faced G indicates the position of 8-OH-Gua. Panel B. 8-OH-Gua-containing ss DNA but labeled at the 3'-end was used. Lane PPRD is piperidine-treated DNA. Other conditions were the same as in Panel A. The position of 8-OH-Gua is indicated by an arrow.

AP DNA was found to be cleaved by such a mild alkali treatment as our sample loading procedure as described in Fig. 2 (Fig. 5A). The position of the band suggests that the product was derived from β elimination reaction of AP



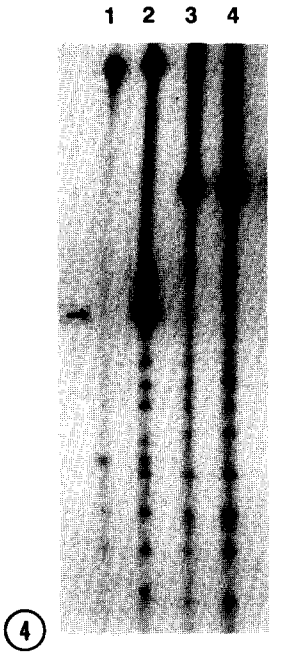


Fig. 3. Decomposition of 8-OH-dG by hot piperidine treatment. 8-OH-dG was treated with piperidine as in Fig. 2 and the reaction mixture was analyzed with HPLC(column: Beckman, Ultrasphere ODS, 5μ; elution: 8% methenol and 10 mM NaH_2PO4). Panel A. Elution profile of 8-OH-dG monitored at 230 nm before (curve a) and after(curve b) treatment with piperidine. 8-OH-dG is detected as peak 1 and its decomposition product as peak 2. The control experiment showed that peak 3 arose from an unknown material present in piperidine solution. Panel B. Spectrum change of 8-OH-dG. The spectra of peak 1(8-OH-dG) and peak 2(decomposition product) are shown by the curves indicated by dashed and dotted lines, respectively.

Fig. 4. Comparison between 8-OH-Gua and other modified purines. Each of the 5'-end labeled ss DNAs was treated with hot piperidine and analyzed on a sequencing gel as in Fig. 2. Lane 1; unmodified 46 mer DNA and lanes 2, 3 and 4; DNAs containing 8-OH-Gua, 8-OH-Ade and O^6-MeGua, respectively. It is noted that because of their smaller size, the bands of 21 mer DNAs containing 8-OH-Ade or O^6-MeGua are located further down than the 46 mer DNA. The cleavage product of ss 46 mer DNA containing 8-OH-Gua is indicated by an arrow.

sites(7). In contrast, the 8-OH-Gua-containing DNA was intact with the same treatment (Fig. 5B). Further, the 8-OH-Gua-containing DNA still remained intact after incubation in 2.0 M glycine-NaOH, pH 12.6 for 2 h at 37°C where most of the AP sites were converted to breaks(1, 10) (data not shown).

DISCUSSION

In the present study, we have found that 8-OH-Gua in DNA is a guanine lesion sensitive to hot piperidine(Fig. 2). When 8-OH-Gua-containing DNA labeled at either the 5'- or the 3'-end was treated with hot piperidine, a scission product appeared at the position of band G in the sequence ladder

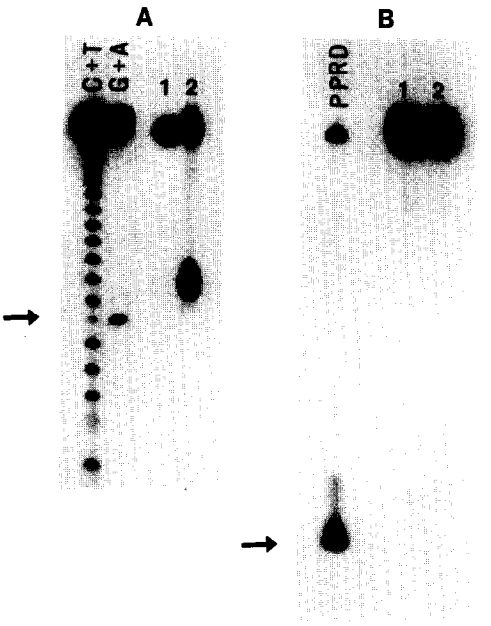


Fig. 5. Difference between 8-OH-Gua and apurinic site. Panel A. AP DNA labeled at the 5'-end was dissolved in the loading buffer used in Fig. 2 and loaded into the gel without(lane 1) and with(lane 2) heating for 3 min at 90°C. Two lanes on the left are the Maxam-Gilbert reactions of the same poly dT but containing dA at the AP site whose position is indicated by an arrow. Panel B. The same experiments were done with 5'-labeled 8-OH-Gua-containing DNA. Lanes 1 and 2 are without and with heating for 3 min at 90°C, respectively, as in Panel A. Lane PPRD; 8-OH-Gua-containing DNA treated with piperidine as in Fig. 2. An arrow indicates the cleavage product.

which indicates the location of 8-OH-Gua(Fig. 2). Under the same conditions, 8-OH-dG was also decomposed(Fig. 3). The nature of the decomposition was not studied further. The finding that the cleavage products of 8-OH-Gua-containing DNA are exactly the same with the G reaction product in the Maxam-Gilbert procedure of the unmodified DNA suggests that there occurs a similar sequence of reactions at the location of 8-OH-Gua as in N^7-methylated guanine; decomposition of the base moiety followed by elimination of both phosphates from the sugar moiety (9).

The hydroxylation on C-8 of adenine and methylation on O^6 of guanine did not render the bases susceptible to hot piperidine(Fig. 4). It appears that among the purine modifications, C-8 hydroxylation of guanine may be a particular lesion which makes the modified moiety labile to base attack and endowed with hot piperidine susceptibility. Further, 8-OH-Gua in DNA is also different from an AP site that is already known as alkali-labile lesion since the former is stable under the usual alkali treatment and can be converted to a strand scission only under drastic conditions like hot piperidine treatment(Fig. 5).

The unique behavior of 8-OH-Gua to hot piperidine can be a useful marker for its identification in DNA. For example, the incorporation of 8-OH-Gua-

containing nucleotide can be easily checked when we intend to synthesize a DNA containing this base at a particular position of the sequence. It was reported that 8-OH-Gua is a major product when DNA was treated with γ-irradiation and systems producing reactive oxygen species(11, 12). Thus, this property of 8-OH-Gua can be useful for estimating the extent of oxidative damage in DNA because the more the damage, the more cleavage will be made by the hot piperidine treatment. It may also be helpful to determine the type of guanine lesion in DNA. For example, formation of 8-OH-Gua can be suspected if a DNA exposed to reactive oxygen species shows cleaved bands at guanine positions on hot piperidine treatment. But in this case, some additional tests may be required for definite identification because other lesions on guanine such as imidazole-ring open form may possibly show the same phenomenon.
The *in vitro* polymerization reaction on the template containing each of the labile guanine residues may be helpful since 8-OH-Gua allows E. coli polymerase I to pass(5) whereas the ring-open form blocks the chain elongation(13).

REFERENCES

1. Sagripanti, J. -L., and Kraemer, K. H. (1989) J. Biol. Chem. 264, 1729-1734.
2. Kasai, H., Crain, P.F., Kuchino, Y., Nishimura, S., Ootsuyama, A., and Tanooka, H.(1986) Carcinogenesis 7, 1849-1851.
3. Kasai, H., Nishimura, S., Kurokawa, Y., and Hayashi, Y. (1987) Carcinogenesis 8, 1959-1961.
4. Kasai, H., and Nishimura, S.(1984) Nucleic acids Res. 12, 2137-2145.
5. Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E., and Nishimura, S.(1987) Nature 327, 77-79.
6. Chung, M. H., Kasai, H., Jones, D. S., Inoue, H., Ishikawa, H., Ohtsuka, E., and Nishimura, S. (1991) Mutation Res. 254, 1-12.
7. Bailly, V., and Verly, V. G.(1987) Biochem. J. 242, 565-572.
8. Tu, C.-P.D., and Cohen, S. N.(1980) Gene 10, 177-183.
9. Maxam, A. M. and Gilbert, W.(1980) Methods in Enzymol. 65, 499-560.
10. Lindahl, T., and Anderson, A. (1972) Biochemistry 11, 3618-3623.
11. Boiteux, S., Gajewski, E., Laval, J., and Dizdaroglu, M. (1992) Biochemistry 31, 106-110.
12. Aruoma, O. I., Halliwell, B., and Dizdaroglu, M. (1989) J. Biol. Chem. 264, 13024-13028.
13. Boiteux, S., and Laval, J. (1983) Biochem. Biophys. Res. Commun. 110, 552-558.