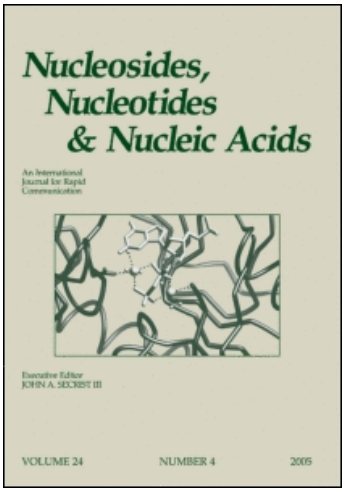

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Novel DNA Damage Mediated by Oxidation of Various Modified Nucleotide Residues

Hideo Inoue[a]; Shiro Koizume[a]; Takahiro Yamauchi[a]; Kouichi Murata[a]; Eiko Ohtsuka[a]
[a] Faculty of Pharmaceutical Sciences, Hokkuido University, Sapporo, Japan

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NOVEL DNA DAMAGE MEDIATED BY OXIDATION OF VARIOUS MODIFIED NUCLEOTIDE RESIDUES

Hideo Inoue,* Shiro Koizume, Takahiro Yamauchi, Kouichi Murata, and Eiko Ohtsuka

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan

ABSTRACT: Permanganate reaction of DNA oligomers containing an 8-oxoadenine or 5-hydroxyuracil residue was studied, and the results were compared with those for an 8-oxoguanine-containing oligomers. We obtained similar results and found that the nucleotide residues neighboring the modified base were damaged and that the novel damage was induced by the oxidation of the modified base.

Permanganate oxidation of DNA is well known as a method for the specific modification of thymine residues within single-stranded DNA.[1] Recently we have carried out this reaction using DNA oligomers with a 7,8-dihydro-8-oxoguanine (8-oxo-G) residue, and found that DNA damage occurs not only at the 8-oxo-G site, but also at the neighboring nucleotide residues.[2] Also, as expected from the unique redox properties of an 8-oxo-G nucleoside,[3] the modified base was more sensitive under the reaction conditions than thymine and the other common bases.

Damaged nucleotide residues were detected by analysis of strand cleavage with hot piperidine treatment or by nucleoside composition analysis of the oxidized oligomers. Investigations, including the oxidation kinetics of 8-oxo-G-containing oligomers, revealed that the reactivity of the common DNA bases around the 8-oxo-G residue is in the order G > A > T, C; similar results were obtained with 8-oxo-G-containing DNA duplexes (manuscript in preparation). This order correlates with the electron donating activities of the bases. These results, combined with the results of the reaction of 8-oxo-G-oligomers with an abasic spacer (manuscript in preparation), suggest that the neighboring nucleotide (base) damage occurs via a redox (electron transfer) reaction between the nucleotides, which is initiated by oxidation of the 8-oxo-G.

Table 1. Sequences, cleavage positions, and percentages of 5'-end-labeled oligodeoxynucleotides

Compound	Sequence	Cleavage yield (%)		
1[2]	5'AT**G**AC**GG**^{ox}AATAT	16	44	25
2	5'AT**G**AC**GGA**^{ox}ATAT	14	40	22
3	5'ATGAC**GGU**^{oh}ATAT	5.4	2.7	48

G^{ox}, A^{ox}, and U^{oh} denote 8-oxo-G, 8-oxo-A, and 5-oh-U, respectively, and bold letters refer to cleavage sites. Cleavage percentages correspond to those for sites with 5' to 3' shown at the left.

8-oxo-G is a common lesion generated by oxidative damage of a guanine residue in DNA. To examine the scope of the permanganate reaction, DNA oligomers with other lesions, 7,8-dihydro-8-oxoadenine (8-oxo-A) and 5-hydroxyuracil (5-oh-U), were tested in this study. We have previously studied the mutagenicity of 8-oxo-G[4,5] and 8-oxo-A[6] residues, which were chemically introduced into DNA oligomers. In this study, we newly synthesized a 5'-DMTr-3'-phosphoramidite building unit for 5-oh-U-modified oligonucleotide synthesis, starting from the selective silyl-protection of the 5-hydroxyl of 5-hydroxy-2'-deoxyuridine. These modified oligomers were prepared on a DNA synthesizer using the standard phosphoramidite method. The strands **1**-**3** were 5'-end-labeled with ^{32}P, and 0.5 μM solutions of the strands were treated with 120 μM $KMnO_4$ in 10 mM sodium phosphate buffer (pH 7.0) for 15 min at 25 °C. In order to examine the damaged positions, the DNAs were subjected to hot alkaline treatment with 1 M piperidine for 30 min at 90 °C, followed by denaturing 20% polyacrylamide gel electrophoresis. The results in Table 1 indicated that damage of the nucleotide (base) residues occurred around the 8-oxo-A and 5-oh-U, as in the case of the 8-oxo-G-oligomer, and that the induction of the damage by the oxidation of the 5-oh-U was weaker than those for the others. It should be noted that the 8-oxo-A site was not cleaved by the oxidation and the piperidine treatment.

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