Adduct formation between oxanine and amine derivatives

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ABSTRACT
Oxanine (Oxa) is a major guanine lesion produced by nitric oxide (NO) under aerobic conditions. To elucidate the genotoxic mechanism of Oxa, this lesion was site-specifically incorporated into an oligonucleotide and allowed to react with cellular amines. Analysis of the reaction product revealed that Oxa formed adducts with spermidine and lysine, suggesting a novel genotoxic mechanism associated with NO-induced DNA damage.

INTRODUCTION
It has been long recognized that chronic inflammation can initiate carcinogenesis. In inflamed tissues, target cells are exposed to excessive amounts of superoxide anion (O$_2^-$) and nitric oxide (NO) liberated from phagocytic cells. Excessive O$_2^-$ and NO may also attack normal cells in proximity, resulting in collateral damage. NO exerts genotoxic effects by complicated mechanisms since NO reacts with O$_2$ and O$_2^-$ yielding N$_2$O$_3$, a powerful nitrosating agent, and peroxynitrite (ONOO$^-$), a powerful oxidizing agent, respectively.

The reaction between DNA and NO under aerobic conditions results in xanthine (Xan), hypoxanthine, and uracil via nitrosation of the primary amino group of G, A, and C, respectively. The reaction with G gives rise to oxanine (Oxa) as the second major product (Xan:Oxa = 3:1, Fig. 1A). Oxa is also formed when DNA or its component is treated with acidic nitrite or N-nitrosoureas. Hypoxanthine and uracil are efficiently removed from DNA by methylpurine glycosylase (MPG) and uracil DNA glycosylase (UDG). Although cells seem to have repair activity for Xan, none of the repair enzymes tested so far exhibited clear activity for Oxa. Accordingly, it is possible that Oxa formed in DNA persists for long time and exerts genotoxic effects. We have previously shown that Oxa forms base pairs with T and C during DNA replication, thus potentially mutagenic. More recently, the deoxyribonucleoside form of Oxa reacts with glycine to form an adduct (Fig. 1B). However, such adduct formation has not been demonstrated in DNA. We report here that Oxa in DNA forms adducts with amines and amino acids.

RESULTS AND DISCUSSION
Deoxyoxanosine 5'-triphosphate (dOTP) was prepared as described previously. A primer for the DNA polymerase reaction was 5'-32P-labeled and annealed to a template. dOTP was incorporated into the defined site of a newly synthesized strand by the DNA
polymerase reaction. The duplex oligonucleotide containing Oxa was incubated with amines and amino acids in Tris-HCl (pH 7.4) at 37°C for varying time. After incubation, products were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE). The amount of products was quantified by a phosphorimaging analyzer.

When the oligonucleotide containing Oxa was incubated with spermidine, a new band indicative of the formation of an Oxa-spermidine adduct appeared in the PAGE analysis (Fig. 1B). The new band migrated slower than the original oligonucleotide. The amount of the adduct increased with incubation time and reached a plateau after 15 min. Incubation with lysine also resulted in a slow migrating band showing Oxa-lysine adduct formation, but the reaction rate was much slower than with spermidine. The amount of the adduct reached a plateau after 48 h of incubation. Adduct formation was not observed when an oligonucleotide containing G in place of Oxa was incubated with spermidine or lysine under the same conditions. Thus, the adduct formation was specific to Oxa and does not occur with intact G. The present results suggest a novel genotoxic mechanism associated with NO-induced DNA damage. If a similar reaction occurs with protein, DNA-protein cross-links will be formed.

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