Mutations induced by glyoxal and methylglyoxal in mammalian cells

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ABSTRACT
To investigate the mutation spectra of glyoxal and methylglyoxal in mammalian cells, we analyzed mutations in a bacterial suppressor tRNA (supF) gene in the shuttle vector plasmid pMY189. The cytotoxicity and the mutation frequency increased according to the doses of glyoxal and methylglyoxal. The majority of glyoxal-induced mutations (65%) were base-pair substitutions, in which G:C—>C:G transversions were predominant. In the mutants induced by methylglyoxal, multi-base deletions were predominant (50%), followed by base-pair substitutions (35%), in which G:C->C:G and G:C—>T:A transversions were predominant.

INTRODUCTION
Glyoxal (GL) and methylglyoxal (MG) are present ubiquitously in beverages and foods, such as coffee, toast, and soy sauce (1,2), as well as in cigarette smoke (3). Moreover, they are formed by lipid peroxidation systems (4,5).

GL and MG are reactive α-ketoaldehydes and form tricyclic compounds with guanine residues in DNA (Figure; 6-9). Thus, these α-ketoaldehydes may induce mutations by the reaction with guanine residues and the subsequent formation of mispairs. Indeed, GL and MG are known to be mutagenic in Salmonella typhimurium strains (1,10). Moreover, we previously reported that GL and MG induce mutations, mainly at G:C pairs in Escherichia coli (11-13). To obtain more knowledge about GL- and MG-induced mutagenesis and to compare the mutation spectra, we analyzed the mutations induced by these compounds in simian kidney (COS-7) cells.

Figure. Glyoxal (R = H) or Methylglyoxal (R = CH₃) adducts with dG.

MATERIALS AND METHODS
Plasmid pMY189 was treated with GL and MG as described, and was immediately transfected into cultured COS-7 cells, as described previously (14). Cytotoxicities and mutation frequencies (MFs) were determined as described (14). The nucleotide sequences of the supF gene fragments were analyzed as described (14).

RESULTS AND DISCUSSION
Glyoxal and methylglyoxal are mutagenic in mammalian cells
The GL- and MG-treated and untreated vectors were transfected into COS-7 cells and were
allowed to replicate in the cells. The plasmid DNAs recovered from the cells were transfected into *E. coli*, and the colonies formed were counted. The relative transforming efficiency is an indicator of cytotoxicity. The number of *E. coli* colonies significantly decreased in GL and MG dose-dependent manners, showing that the replication was partially blocked by the GL- and MG-adducted residues.

The plasmid DNAs recovered from the cells were also transfected into the indicator *E. coli* to obtain the *supF* mutants. The mutation frequency increased according to the doses of GL and MG.

**Glyoxal- and methylglyoxal-induced mutation spectra**

In the mutants induced by GL, base-pair substitutions were predominant (65%), followed by multi-base deletions (28%, the range of deleted sequences was 29-233 bp). Among the base-pair substitutions, 89% of the mutations occurred at G:C pairs, and G:C→T:A transversions were predominant (44% of the base-pair substitutions), followed by G:C→A:T transitions (25%), G:C→C:G transversions (19%), and A:T→T:A transversions (11%).

In the mutants induced by MG, multi-base deletions were predominant (50%, the range of deleted sequences was 8-320 bp), followed by base-pair substitutions (35%). Among the base-pair substitutions, 89% of the mutations occurred at G:C pairs, and G:C→C:G and G:C→T:A transversions were predominant (39% and 33% of the base-pair substitutions), followed by G:C→A:T transitions (17%), and A:T→G:C transitions (11%).

Thus, the presence of the methyl group may alter the DNA polymerase recognition patterns of the base pairs involving tricyclic adducts and/or other adducts.

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**REFERENCES**