MECHANISM OF MUTAGENESIS BY *N*-METHYL-*N'*-NITRO-*N*-NITROSO-GUANIDINE (MNNG) V. METHYLATION OF DNA BY *N*-TRIDEUTERIOMETHYL-*N'*-NITRO-*N*-NITROSO-GUANIDINE (D_3-MNNG)*

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1. Introduction

The mutagenic effect of MNNG is considered to be in part due to methylation of nucleic acids [1–7]. The formation of diazomethane as an intermediate has been suggested [1, 2]. There are some observations which do not support diazomethane as an intermediate [6, 8]. In an attempt to answer this question, we have studied the methylation of DNA by D_3-MNNG *in vitro*.

2. Materials and methods

D_3-MNNG was synthesized from trideuteriomethyl ammoniumchloride (Merck) and nitroguanidine (EGA) by the method of McKay [9]. High molecular weight DNA (EGA) (800 mg/l) was incubated with D_3-MNNG (400 mg/l) at 37° for 2 days in phosphate-citric acid buffer (pH 6.0). The reaction mixture was flash-evaporated, hydrolyzed in 1 N HCl, and chromatographed on a column of Dowex W 50 X 8 (200–400 mesh). Elution was performed with a linear gradient of 1 N to 4 N HCl according to the method of Magee [10]. 7-Methylguanine was further purified by chromatography on Sephadex CM-25 and finally crystallized from methanol. The deuterium content was determined by mass spectrometry.

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3. Results and discussion

The mass spectrum of isolated 7-methylguanine has a parent peak at *m/e* 168, which corresponds to 7-trideuteriomethyl-guanine. High resolution mass spectroscopy resulted in the molecular formula $C_6H_4D_3N_5O$. Dideuterio-diazomethane (CD_2N_2) as an intermediate of the methylation reaction would result in the molecular formula $C_6H_5D_2N_5O$ with a parent peak at *m/e* 167. From this result we conclude that the mechanism of methylation *in vitro* at pH 6.0 does not involve the formation of diazomethane as an intermediate. We suggest that the methyl group is transferred as an intact unit.

On the basis of this result, we assume that also the methylation of the nucleic acids by MNNG *in vivo* does not involve diazomethane as an intermediate. This is in agreement with results of Lijinsky et al. [11] who studied the methylation of RNA and DNA of rat liver by feeding di(trideuterio)methylnitrosamine and who isolated 7-trideuteriomethylguanine as a reaction product.

The rate of methylation of nucleic acids by MNNG *in vitro* is enhanced by sulfhydryl compounds [6]. Presumably an activation by sulfhydryl compounds also takes place in the cell.

Experiments on methylation by D_3-MNNG of DNA *in vitro* in the presence of sulfhydryl compounds and of nucleic acids in cells of *Escherichia coli* are in progress.

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