MECHANISM OF MUTAGENESIS BY *N*-METHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE (MNNG)*. METHYLATION OF NUCLEIC ACIDS BY *N*-TRIDEUTERIOMETHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE (D_3-MNNG) IN THE PRESENCE OF CYSTEINE AND IN CELLS OF *ESCHERICHIA COLI***

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Received 5 February 1971

1. Introduction

The mutagenic effect of MNNG is considered to be in part due to methylation of nucleic acids. The formation of diazomethane as an intermediate of methylation of DNA by D_3-MNNG in vitro at pH 6.0 can be excluded by our former results [1]. As the rate of methylation of nucleic acids is enhanced by sulfhydryl compounds [2], we studied the mechanism of methylation of DNA by D_3-MNNG in vitro in the presence of cysteine. Furthermore, we studied the mechanism of methylation of nucleic acids by D_3-MNNG in vivo in *Escherichia coli* B.

2. Materials and methods

D_3-MNNG was synthesized from trideuterio-methyl-ammoniumchloride (Merck) and nitroguanidine (EGA) by the method of McKay [3].

a) High molecular DNA (EGA) (800 mg/l) was incubated with D_3-MNNG (400 mg/l) and cysteine-hydrochloride (770 mg/l) at 37° for 2 days in phosphate–citric acid buffer (pH 6.0). 7-Methyl-guanine was isolated and purified as described previously [1] and the deuterium content was determined by mass spectrometry.

b) Freshly prepared and washed cells of *Escherichia coli* B (100 g) were resuspended by stirring in 8 l of saline–phosphate–citric acid buffer (6.8×10^9 cells per ml) and incubated at 20° for 60 min with D_3-MNNG (4.3 g/l). The cells were then centrifuged at 6500 *g*, washed twice with saline and finally frozen at −20°.

On the next day each group consisting of 8 g cells was resuspended in 50 ml of saline–EDTA (pH 8.0) with 2% laurylsulfate and homogenized with 15 g Ballotini (diameter 0.1 mm) in a Braun-homogenizer (Melsungen) for 6 min at a temperature of 0°. The suspension was centrifuged at 35,000 *g* for 20 min and the supernatant was saved. This procedure was repeated twice. The nucleic acids were isolated from the combined supernatants by the method of Marmur [4], finally hydrolyzed in 300 ml of 1 N HCl at 80° for 80 min and 7-methylguanine was isolated and purified [1]. The deuterium content was then determined by mass spectrometry.

3. Results and discussion

The mass spectra of the isolated 7-methylguanine received from the described experiments in vitro and in vivo have a parent peak at m/e 168, according to 7-trideuterio-methyl-guanine. From this result we conclude that diazomethane (CD_2N_2) is neither formed as an intermediate in reaction of MNNG with DNA in the presence of SH-compounds in vitro

* Part VI.

** In part presented by R.Süssmuth in August 1970 at the 1st International Symposium of Genetics of Industrial Microorganisms in Prague.

nor in reaction of MNNG with cells of *E. coli* B in vivo at pH 6.0. It seems therefore, that the methyl group is transferred as an intact unit. According to our former experiments in vitro [2], it seems that an activation of methylation by methionine also plays an important role. The methylation rate of nucleic acids by MNNG is increased in the presence of methionine. This could suggest that not only the methyl group of MNNG is transferred to the nucleotides but also the methyl group of methionine.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We express our thanks to Mr. G.Nicholson (Tübingen) and Dr. K.Frei and Dr. H.Lichti, Sandoz AG (Basel) for the mass spectra.

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