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MODULATION OF CARCINOGEN CHROMATIN-DNA INTERACTION BY POLYAMINES

S. Rajalakshmi, Prema M. Rao and D.S.R. Sarma

Department of Pathology, University of Toronto,
Medical Sciences Building
Toronto, Ontario M5S 1A8, Canada

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SUMMARY: The methylation of rat liver chromatin DNA has been studied in vitro by the direct-acting carcinogen N-methyl N-nitrosourea. It is shown that spermine inhibits the methylation of chromatin DNA at the N^7 and O^6 positions of guanine and the N^3 position of adenine. However, spermine does not inhibit the methylation of 2-deoxy-5'-guanilic acid included as an internal control in the reaction. Under the experimental conditions, spermine exerts no influence on the degradation of N-methyl N-nitrosourea. The study has revealed that compounds like spermine or spermidine which bind tightly to DNA can modulate carcinogen-DNA interaction either by altering the net charge and/or the conformation of DNA.

INTRODUCTION:

Polyamines appear to play a key role in the regulation of cellular metabolism. The transition of many mammalian cells from a relatively quiescent to a rapidly proliferating state is accompanied by an increase in the synthesis of polyamines (1-3). Polyamines have high affinity for nucleic acids by virtue of their polybasic nature. They bind tightly to the phosphate residues and reduce the net negative charge of DNA, and also exert a stabilizing influence on its structures (1,4-6). The conformation nucleic acids assume in the presence of polyamines is stimulatory for macromolecular synthesis, such as DNA synthesis and DNA dependent RNA synthesis (1,7-10). In view of these effects of polyamines on DNA structure and its function, it was of interest to determine whether polyamines by so stabilizing the structure of DNA and neutralizing the charges on it will modulate DNA-carcinogen interaction.

For this purpose, the influence of polyamines on the methylation of chromatin DNA in vitro by N-methyl N-nitrosourea (MNU) was studied. The study

Abbreviations: MNU, N-methyl N-nitrosourea; PCA, perchloric acid.

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reveals that in contrast to the reported stimulatory effect of polyamines on enzymatic reactions involving DNA, polyamines inhibit the chemical methylation of chromatin DNA by MNU. The results indicate that carcinogen-DNA interaction can be modulated by compounds that affect either the net charge and/or the structure of DNA.

MATERIALS AND METHODS

MNU (K and K Fine Chemicals, Plainview, N.Y.) was recrystallized using ethylacetate and hexane. The radiopurity of [^{14}C-methyl]-MNU (New England Nuclear, Boston) was checked using high pressure liquid chromatography on a µbondapak C-18 column and 0.05M ammonium formate pH 5.0 as the eluting buffer. Polyamines were obtained from Sigma Chemicals, St. Louis, Mo.

Alkylation of rat liver chromatin-DNA with [^{14}C]-MNU

Hepatic chromatin was isolated from male Fischer rats (120-150 g) by methods described in our previous publication (11). Chromatin (150-200 µg DNA/ml) suspended in 0.01M glycine was incubated in a total volume of 4 ml of 0.01M Tris-HCl pH 7.8 with [^{14}C]-MNU (2 µmoles/ml; sp.act. 0.9 µCi/µmole) for 2 hr. The pH was maintained constant throughout at 7.8. The A_{260nm} and A_{390nm} at the end of incubation showed complete degradation of MNU. The nucleoprotein was precipitated with cold perchloric acid (0.5N final concentration) and washed repeatedly with cold 0.25N PCA. It was suspended in 2 ml of 0.01M Tris-HCl pH 7.2 and 0.5% sodium dodecylsulfate and deproteinized with Proteinase K (200 µg/ml) at 37°C for 15 hr. The DNA was precipitated with 0.5N PCA in the cold and recovered by centrifugation. The alkylated bases in the DNA were released by mild acid hydrolysis (0.1N PCA) at 70°C for 45 min. The absorbance at 260 nm was measured in a Beckman spectrophotometer and the radioactivity determined in an Intertechnique scintillation spectrometer.

RESULTS AND DISCUSSION

MNU was employed in this study, since it requires no metabolic activation prior to its interaction with DNA, RNA or protein (12).

The results described in Table I reveal that addition of 0.0125, 0.025 and 0.05 µmoles of spermine per ml of incubation mixture containing 150-200 µg of chromatin DNA inhibits the methylation of DNA by 27, 56 and 86% respectively. Similar patterns of results were obtained using rat liver DNA instead of liver chromatin as the substrate. Spermidine exerted a similar effect, while putrescine was less effective (50% inhibition at 0.1 µmole/ml) in inhibiting the methylation reaction.

The observations recorded in Table II reveal that the formation of N^7 methylguanine (N^7meG), O^6methylguanine (O^6meG) and N^3 methyladenine (N^3meA) are all affected by polyamines. Interestingly, at about 86% inhibition, the

TABLE I

Effect of spermine on the methylation of chromatin-DNA by MNU

Spermine (μmoles/ml incubation mixture)	% Inhibition
0	-
0.0125	27 ± 0.88 *
0.0250	56 ± 1.50
0.050	86 ± 0.67

* mean ± S.E. of six experiments

The specific activity of control methylated chromatin-DNA where spermine was not added was 14400 ± 632 DPM per $A_{260}{}_{nm}$

methylation at N^3 position of adenine was inhibited by 100% and those of N^7 or O^6 position of guanine were inhibited by 80%.

Polyamines can inhibit DNA methylation by MNU either by inhibiting the release of active methyl groups from MNU during its decomposition at pH 7.8 and/or by interacting with the phosphate residues of DNA, they can alter the net charge and conformation of DNA such that methylatable sites are not available for MNU.

In order to determine whether polyamines would influence the rate of degradation of MNU under our experimental conditions, the effect of spermine on the decomposition of MNU was studied. Polyamines had no influence on the degradation of MNU at pH 7.8, as measured by absorption at 390 and 260 nm. Furthermore, the inclusion of 2-deoxy-5'-guanylic acid (dGMP) in the reaction mixture as an internal control showed that polyamines exerted no effect on dGMP methylation (results not included). This fact indicates that the inhibition of MNU-induced methylation of DNA by polyamines is not due to a suppression of release of active methyl groups from MNU during its decomposition at pH 7.8.

TABLE II

Effect of spermine on the formation of N^7meG, O^6meG and N^3meA in liver chromatin-DNA by MNU

Spermine (μmoles/ml of incubation mixture)	Percent inhibition in			
	Total methylation	N^7meG	O^6meG	N^3meA
0.025	56	60	50	63
0.050	86	80	80	100

The alkylation reaction was carried out as described in the text. The alkylated bases were fractionated on Sephadex G-10 using 0.05M ammonium formate pH 6.8 as the eluting buffer (13). The peaks corresponding to standard N^7-meG, O^6meG and N^3meA were pooled, lyophillized, dissolved in 0.1N HCl and radioactivity was determined. The experiment was repeated two times and similar patterns of results were obtained.

The major site of interaction of MNU with DNA bases is at the N^7 position of guanine (12). In a space filling model of DNA in the B-form, the N^7 position of guanine can be seen in close proximity to the PO_2^{--} anions of the backbone of DNA (14). Polyamines, by virtue of their polycationic nature, bind non-covalently to the phosphate residues of DNA and neutralize their negative charge (4). This, in turn, might reduce the nucleophilicity of the N^7 position of guanine and other sites susceptible for methylation in the DNA by MNU. Alternatively, the neutralization by polyamines of phosphate charges which usually repel each other may lead to an increase in the net strength of various cohesive forces, such as Van der Wall forces and hydrogen bonding, stabilizing the secondary structures like the double helix. The complex formed between spermine and DNA is characterized by regular conformation with the elimination of defects, which in aqueous solution are very likely to be concentrated in those regions of DNA rich in the more solvated A-T base pairs (4). In such an organized helical conformation, the methylatable sites on DNA by MNU are probably rendered unavailable for interaction with

MNU. It is of interest to note that the ordered structure DNA assumes in the presence of spermidine does not permit the intercalation of 3,4-benzpyrene(15).

Even though the mechanism by which polyamines inhibit alkylation of DNA by MNU is not known, the results of this communication focus our attention on the fact that certain conformation of DNA may be required for carcinogen-DNA interaction. It would be of interest to study in future whether the effect of polyamines on carcinogen-DNA interaction is restricted to methylating agents like MNU and polycyclic hydrocarbons like 3,4-benzpyrene, or is common to all carcinogens.

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