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Action of spermidine, N^1-acetylspermidine, and N^8-acetylspermidine at apurinic sites in DNA

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The cleavage efficiency of spermidine and its acetyl derivatives (N^1-acetylspermidine and N^8-acetylspermidine) at apurinic sites in DNA were examined by PAGE-urea analysis. The three polyamines induced different rates of cleavage when compared at 1 mM concentrations. The order of effectiveness were: spermidine > N^8-acetylspermidine > N^1-acetylspermidine. Thus a decrease in efficiency was observed when the first order amino-groups of spermidine were blocked. The N-[8]amino-group of spermidine was less effective in inducing cleavage at AP-sites than the N^1-amino-group. Among several proposed models of polyamine-DNA interactions, our results can best be explained by the model postulated by Liquori et al. [6].

Apurinic/apyrimidinic (AP) site; Polyamine; DNA repair

1. INTRODUCTION

Putrescine, spermidine, spermine and closely related derivatives are present up to millimolar concentrations in all living matter tested [1,2]. Evidence accrued over the years strongly support the view that adequate levels of polyamines are necessary for the cell proliferation and differentiation [3-5]. Furthermore, the interaction of polyamines with nucleic acids are believed to be essential for realization of the physiological functions of polyamines. Polyamines appear to be present in the nucleus in quantities sufficient to neutralize 15-30% of the DNA negative charges [12]. The existence of specific [6] and nonspecific [7] interactions between DNA and polyamines have been postulated.

Polyamines and their acetyl derivatives stabilize DNA and nucleosomes against thermal and X-ray induced denaturation [8,9], enzymatic cleavage [10] and induce aggregation of DNA [11]. They may also protect the DNA against alkylating agents [26]. In bacteria, DNA is packed by polyamines, non-histone proteins and RNA [13]. Viral genomes are also compacted by polyamines and viral proteins [14]. In eukaryotes, endogenous polyamines are associated with highly condensed chromatine in vivo [15]. Furthermore, increasing conversion of B-DNA to Z-DNA in the presence of low concentration of polyamines has been observed [16].

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Polyamines are also known to affect the activities of the enzymes involved in nucleic acid metabolism like DNA polymerases, DNA ligases, apurinic/apyrimidinic (AP) endonucleases, polynucleotide kinase, UV-endonucleases [17], and 3-methyladenine-DNA glycosylases [18] and prokaryotic and eukaryotic topoisomerases [19]. In 1972, Lindahl and Andersson [25] showed that putrescine induced cleavage at AP-sites in DNA, and later by Male et al. [20] that polyamines were able to catalyze cleavage of phosphodiester linkages at AP-sites in supercoiled, relaxed, and denatured DNA and also in core and chromosome particles. The order of effectiveness of polyamines were established as follows: spermine > spermidine > putrescine > cadaverine [20]. Several models have been proposed for the interaction between DNA and polyamines, and conflicting results have been obtained [6,26,27,30].

In the present report we have examined and compared the cleavage efficiency of spermidine, N^1-acetylspermidine and N^8-acetylspermidine at apurinic sites in DNA, and applied the results to propose the most probable model for spermidine-DNA interactions.

2. MATERIALS AND METHODS

2.1. *Chemicals, polyamines and enzymes*

Ultrapure urea, acrylamide and methylenebisacylamide were purchased from Bethesda Research Laboratories. Spermidine (*N*-[3-aminopropyl]-1,4-butanediamine), N^1-acetylspermidine, N^8-acetylspermidine and piperidine were obtained from Sigma. The DNA-polymerase I (*E.coli*) 'Klenow large fragment' was from Amersham International and *Sal*I and *Hae*II restriction endonucleases were from New England Biolabs.

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2.2. *Preparation of DNAs*

The plasmid pUC18 was isolated and purified as described by Maniatis et al. [21]. The plasmid was digested with *SalI* restriction endonuclease, and 3′-endlabeled with [α-^{32}P]dNTP's and the 'Klenow large fragment'. A subsequent digest with *Hae*II restriction endonuclease resulted in two labeled DNA fragments, 182 and 263 nucleotides in length.

2.3. *DNA modification*

The apurinic sites were introduced in the two ^{32}P-labeled pUC18 fragments by formic acid treatment. Approximately 0.5 μg of DNA was incubated with 70% formic acid at 22°C for 5 min [22]. This treatment introduced approximately one apurinic site per labeled DNA-strand, randomly distributed.

2.4. *Assays conditions*

The standard assay condition for the polyamines was 10 mM Na_2HPO_4/NaH_2PO_4, 2 mM EDTA, pH 8.0. If not otherwise stated, the polyamine concentration was 1 mM. Incubation was performed at 37°C for 20 min, and terminated by phenol extraction.

2.5. *DNA sequencing analysis*

The chemical modification DNA sequencing procedure described by Maxam and Gilbert [22] was employed. The Gua- and the Ade + Gua lanes were run alongside the analysis lanes to verify the cleavage sites. The Ade + Gua lane contained the apurinic DNA employed in the analysis, but cleaved chemically by piperidine. The DNA sequencing analysis was performed as described previously [23,24] and the autoradiograms obtained were densitometer scanned to compare the rate of cleavage induced.

3. RESULTS

DNA fragments from the plasmid pUC18, 3′-labeled and with approximately one AP-site per labeled strand, were employed in the analysis. The cleavage products created by the action of spermidine, N^1-acetylspermidine, and N^8-acetylspermidine, were analysed on DNA sequencing gels. To verify the polyamine-induced cleavage sites, the Gua and/or the Ade + Gua DNA sequencing lanes were run alongside the analysis lanes. The autoradiograms obtained were densitometer scanned to compare the rate of cleavage.

The cleavage patterns created by the three polyamines using the 262 bp 3′-labeled pUC18 *SalI/Hae*II restriction endonuclease fragment as substrate, are displayed in Figs 1 and 2. Three concentrations of the respective polyamines were compared. For all three polyamines, 1 mM concentration induced a higher rate of cleavage than 0.01 mM and 0.1 mM. The 0.01 mM lanes were approximately at the level of the background (Figs 1 and 2), and the 0.1 mM lanes just above this level (Fig. 1). We have therefore chosen the 1 mM concentration to compare the rate of cleavage (Table I). It is obvious from the autoradiograms in Figs 1 and 2, that the apurinic DNA treated with 1 mM spermidine has been cleaved more frequently than DNA treated with N^1-acetylspermidine or N^8-acetylspermidine. Furthermore, 1 mM N^8-actylspermidine induced a higher rate of cleavage than 1 mM N^1-acetylspermidine.

The cleavage pattern of the three polyamines at 1 mM concentrations, using the 182 bp 3′-labeled pUC18

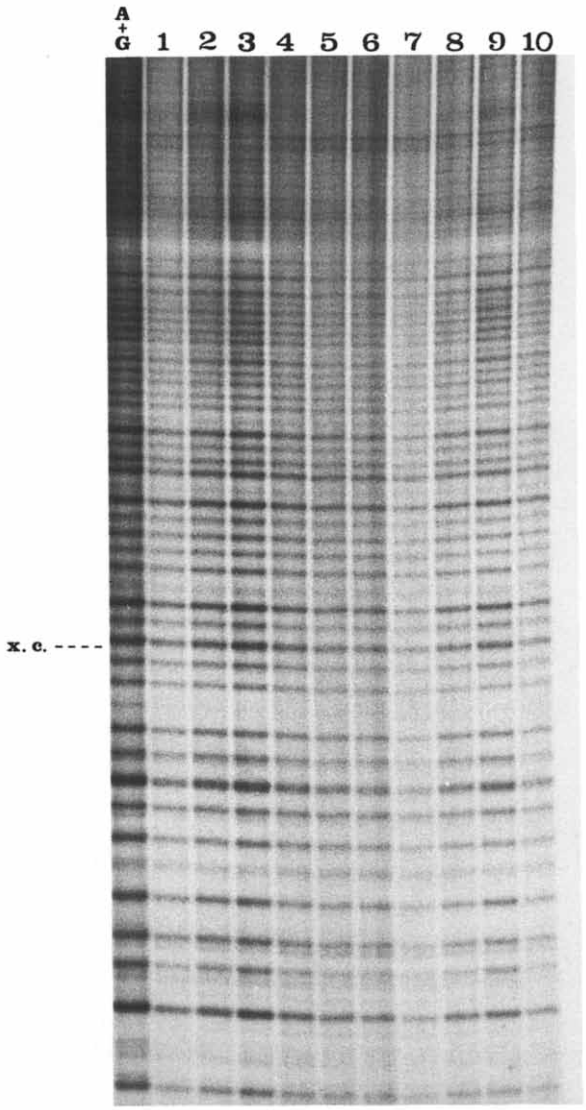

Fig. 1. The cleavage patterns of the three different polyamines examined at apurinic sites in the 263 bp 3′-labeled pUC18 *SalI/Hae*II restriction endonuclease digest are displayed. The DNA fragments were separated on a 20% DNA sequencing gel. A + G lane: Ade and Gua specific sequencing lane. Lanes 1–10 contained ^{32}P-labeled DNA with apurinic sites. Lanes 1–3 were incubated with spermidine, lanes 4–6 incubated with N^1-acetylspermidine, and lanes 7 through 9 were incubated with N^8-acetylspermidine. Lane 10, the control lane, was incubated without polyamines. Lanes 1, 4 and 7 contained 0.01 mM concentrations, lanes 2, 5 and 8 contained 0.1 mM concentrations and lanes 3, 6 and 9 contained 1 mM concentrations of the respective polyamines.

*SalI/Hae*II fragment, are displayed in Fig. 3. The results displayed shows that spermidine induced a higher rate of cleavage than the other two polyamines. It is also obvious that N^8-acetylspermidine has a higher cleavage efficiency than N^1-acetylspermidine. These results are in full agreement with the results in Figs 1 and 2, compared in Table I, and from several other autoradiograms not shown.

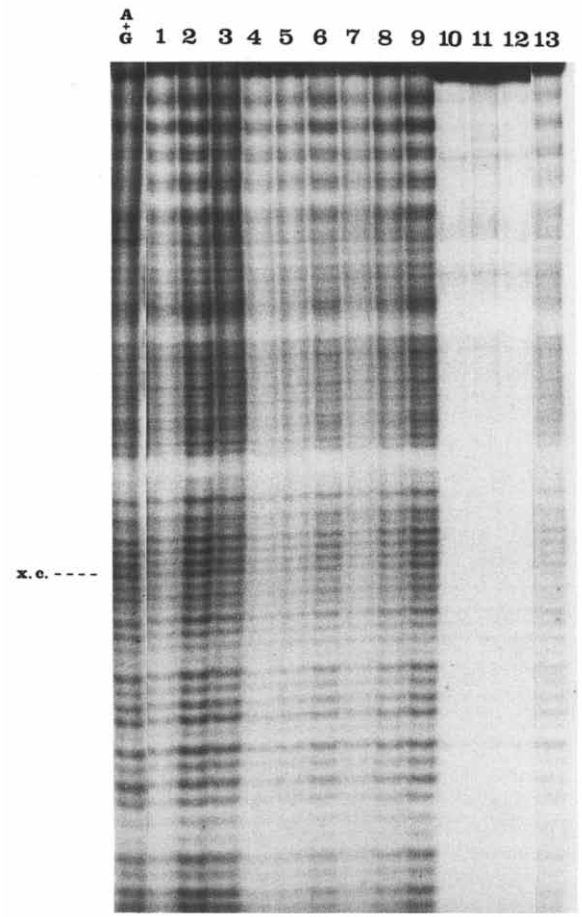

Fig. 2. Autoradiogram of a 20% DNA sequencing gel showing the action of spermidine, N^1-acetylspermidine, and N^8-acetylspermidine at apurinic sites in the 3'-labeled 263 bp pUC18 *SalI/HaeII* restriction endonuclease fragment. A + G lane: Ade and Gua specific sequencing lane. Lanes 1 through 9 and lane 13 contained apurinic DNA. In lanes 1–3, the apurinic DNA was incubated with spermidine, in lanes 4–6 incubated with N^1-acetylspermidine, and in lanes 7–9 with N^8-acetylspermidine. Lanes, 1, 4 and 7 contained 0.01 mM concentrations, lanes 3, 5 and 8 contained 0.1 mM concentrations and lanes 2, 6 and 9 contained 1 mM concentrations, of the respective polyamines. Lane 13, a control lane, contained apurinic DNA incubated without polyamines. Lanes 10–12 contained unmodified DNA, incubated with 1 mM spermidine in lane 10, with 1 mM N^1-acetylspermidine in lane 11, and with 1 mM N^8-acetylspermidine in lane 12.

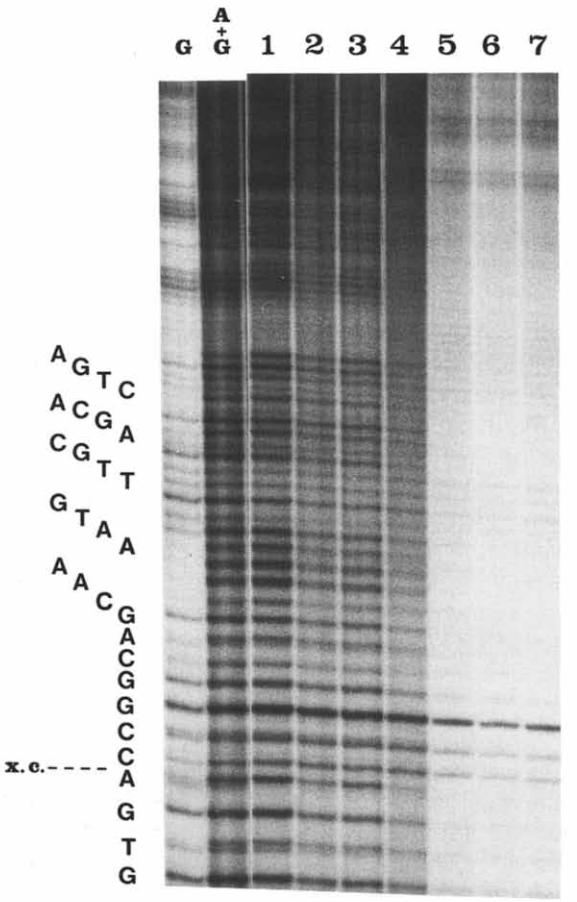

Fig. 3. Autoradiogram of a 20% DNA sequencing gel displaying the cleavage patterns of spermidine, N^1-acetylspermidine, and N^8-acetylspermidine at apurinic sites in the 3'-labeled 182 bp pUC18 *SalI/HaeII* restriction endonuclease fragment. G: Gua specific sequencing lane. A + G: Ade and Gua specific sequencing lane. Lane 1 contained apurinic DNA incubated with 1 mM spermidine, lane 2 contained apurinic DNA incubated with 1 mM N^1-acetylspermidine, and lane 3 contained apurinic DNA incubated with 1 mM N^8-acetylspermidine. Lanes 5 through 7 contained unmodified DNA, incubated with 1 mM spermidine in lane 5, with 1 mM N^1-acetylspermidine in lane 6, and with 1 mM N^8-acetylspermidine in lane 7. Lane 4 contained apurinic DNA incubated without polyamines.

Table I

The three autoradiograms displayed in Figs 1–3 were densitometer scanned and the relative amount of nicking (%) was measured. The Ade + Gua lanes in the respective autoradiograms were set to 100% and the respective substrate lanes, the backgrounds, were substracted. The lanes with 1 mM concentration of the respective polyamines, were compared in % relative to the Ade + Gua lane. Since the amount of radioactivity loaded in each lane varied somewhat, the values measured had to be adjusted relative to the amount loaded. Sd, 1 mM spermidine; N^1-AcSd, 1 mM N^1-acetylspermidine; N^8-AcSd, 1 mM N^8-acetylspermidine.

Autoradiogram	Sd	N^1-AcSd	N^8-AcSd
1	78	12	31
2	105	28	67
3	88	21	46

4. DISCUSSION

The effect of polyamines on apurinic/apyrimidinic (AP) DNA has been investigated previously by several investigators [25,20], and the cleavage induced is most probably caused by β-elimination [25,31]. We have compared the efficiency of three different polyamines which share a common structure. By comparing the rate of cleavage induced at apurinic sites for spermidine (Fig. 4) and derivatives of spermidine where the respective amino-groups were modified to acetyl-groups, we have studied the influence of the different amino-groups on the phosphodiester bond cleavage.

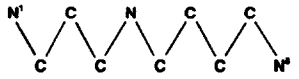

Fig. 4. Schematic model of spermidine.

The rate of cleavage at AP-sites has been shown to be proportional to the concentration of polyamines up to the order of magnitude of 1 mM [20]. At higher concentrations, aggregations of DNA have been found [11]. The efficiency of polyamine induced cleavage in the aggregates was similar to free DNA, but aggregates of DNA will disturb the separation on a DNA sequencing gel. We have therefore chosen to compare concentrations up to 1 mM. Concentrations lower than 1 mM were in several of the autoradiograms displayed close to the background level. The concentration of 1 mM was therefore chosen. The results from nine autoradiograms, three of them displayed in the present paper, strongly suggest that the order of effectiveness in inducing phosphodiester bond cleavage at apurinic sites were: spermidine > N^8-acetylspermidine > N^1-acetylspermidine. The two first order amino-groups of spermidine were not equivalent in inducing DNA cleavage. Most effective was the amino-group attached to the aminopropyl part of spermidine (N^1) compared with the amino-group of the tetramethylen carbon chain of spermidine (N^8) derived from putrescine, which was less effective.

Several models have been proposed for the interaction between DNA and polyamines, and conflicting results have been obtained [6,27-30]. Theoretically, polyamines can interact with DNA in several ways: within the minor or the major groove, along the phosphate backbone, or across the major or the minor groove. Our results support the model proposed by Liquori et al. [6]. This model assumes that the secondary amino-group of spermidine and the amino-group attached to the aminopropyl part of spermidine (N^1) interact with the same single polynucleotide chain, when the amino-group of the tetramethylen carbon chain of spermidine (N^8) interacts with the opposite polynucleotide chain (Fig. 5). Thus the secondary and the N^1-amino-group of spermidine might have a larger influence on the phosphodiester bond cleavage than the N^8-amino-group because they interact with the same polynucleotide chain and will thereby form a more stable complex with this chain. The tetramethylen carbon chain bridges the gap of the narrow groove between the two polynucleotide chains, allowing the N^8-amino-group to interact with the opposite chain. This interaction is an interaction between one amino-group (N^8) and one phosphate group, and might not be as stable as the interaction with the first polynucleotide chain.

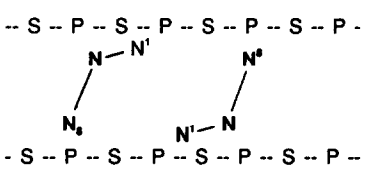

Fig. 5. Schematic model showing the amino-groups of two spermidine molecules interacting with the phosphate-groups of the two single polynucleotide chains of the double helix. The bases are omitted to simplify the model [25]. S, deoxyribose of DNA; P, phosphate-group of DNA; N, amino-group of spermidine.

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