

Inhibition of the activity of restriction endonucleases by spermidine and spermine

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Physiological concentrations (0.5–2.0 mM) of spermidine and spermine were observed to inhibit the digestion in vitro of plasmid pJDB 207 by the restriction endonucleases *Bam*HI (EC 3.1.23.6), *Eco*RI (EC 3.2.23.13), *Hind*III (EC 3.1.23.20), *Hpa*I (EC 3.1.23.23) and *Pst*I (EC 3.1.23.31). The polyamines protected all the tested restriction sequences of DNA, since the activity of all endonucleases used was strongly inhibited. These results show the need for caution when using polyamines as experimental tools for recombinant DNA chemistry.

<i>Endonuclease</i>	<i>Polyamine</i>	<i>Plasmid digestion inhibition</i>
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1. INTRODUCTION

There are abundant reports in the literature indicating that polyamines are essential compounds for cell growth and proliferation [1,2]. One explanation for the need of polyamines in cell growth is the binding of polycationic polyamines to negatively charged phosphate groups of DNA [3–6]. This binding could lead to the neutralization of highly charged DNA, making it more suitable for the compact packing and folding occurring in the cell.

Besides interacting directly with DNA it has been shown that polyamines stimulate the activity of DNA gyrase [7], DNA polymerase [8] and DNA ligase [9]. This suggests that polyamines may change the conformation of DNA to form structures which are more suitable for enzymatic reactions concerned with DNA metabolism or, as shown here, change the characteristics of plasmid DNA so that it is less suitable for endonucleolytic cleavage in vitro than unmodified DNA.

2. MATERIALS AND METHODS

2.1. Materials

The polyamines, putrescine, spermidine and spermine (as their hydrochlorides) were purchased from Sigma (St. Louis, MO). Restriction enzymes were obtained from Boehringer (Mannheim, FRG).

Plasmid pJDB 207 (obtained from Dr Beggs) was kept in *Escherichia coli* JA 221. To obtain large amounts of plasmid DNA for restriction studies, *E. coli* JA 221 was grown in L-broth medium in the presence of ampicillin (50 µg/ml) and plasmid DNA was extracted from overnight cultures and purified by banding in CsCl/ethidium bromide gradients by the method of [10].

2.2. Methods

Restriction enzyme reactions were carried out in a reaction mixture at 37°C as recommended by the manufacturer (Boehringer; Catalog; Biochemicals for Molecular Biology). The amounts of restriction enzyme for each assay were 5 units of *Bam*HI, *Hind*III, *Hpa*I or *Pst*I and 5–75 units of *Eco*RI. The incubation time for each reaction was 60 min,

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and the reaction was stopped by heating at 65°C for 10 min. When spermidine or spermine were not present from the beginning of the incubation, one or the other was always added to the samples at a final concentration of 1 mM immediately before heating, to control any possible effects of polyamines on the migration of DNA during the gel electrophoresis.

The heated DNA samples were subjected to electrophoresis in 0.6% agarose slab gels, containing 90 mM Tris/boric acid and 2.5 mM EDTA, pH 8.2. Constant voltage (1.5 V/cm of gel) was used and the running time was 16 h. After the run DNA bands were visualized by staining with ethidium bromide.

3. RESULTS

3.1. Effects of spermidine on endonucleolytic cleavage of plasmid pJDB 207

Plasmid pJDB 207 has only one cutting point for each of the restriction enzymes *Bam*HI, *Hind*III, *Hpa*I and *Pst*I and when this plasmid is linearized by any of these endonucleases it yields a 6.9 kb DNA fragment [11]. *Eco*RI cuts at 3 points and under normal conditions it yields 3 DNA fragments from pJDB 207 with values of 3.6, 2.5 and 0.8 kb. Fig.1 shows that 1 mM spermidine inhibited totally the activity of *Hind*III (lane 7) and *Hpa*I (lane 11) endonucleases and partially the reaction catalyzed by *Bam*HI (lane 8) and *Pst*I (lane 10). In the case of *Eco*RI (lane 9) spermidine modulated the reaction giving several new DNA fragments which were not seen in the control sample (lane 3). Digestion with *Eco*RI in the presence of spermidine produced 3 new fragments (band nos.3,4 and 6 from the top of fig.1, lane 9) with sizes (estimated from their electrophoretic mobilities) of 6.1, 4.4 and 3.3 kb, respectively. Presumably these new fragments correspond to partial digestion products (3.6 + 2.5, 3.6 + 0.8, and 2.5 + 0.8, respectively) in each of which a different one of the possible cleavage sites has not been cut. Although the 3.6 and 2.5 kb limiting digestion products can also be seen in lane 9 of fig.1, the 0.8 kb band fragment was apparently formed in amounts too small to detect (some is expected, however, to complement the 6.1 kb partial digestion fragment).

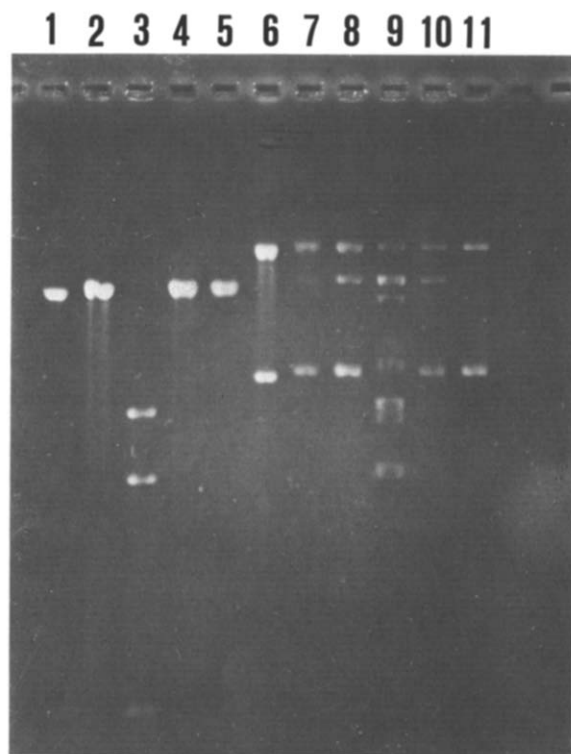


Fig.1. Effect of 1 mM spermidine on the digestion of pJDB 207 by various restriction endonucleases. 1.5 µg of plasmid DNA was digested as described in section 2 as follows: lane 1, *Hind*III; lane 2, *Bam*HI; lane 3, *Eco*RI; lane 4, *Pst*I; lane 5, *Hpa*I; lane 6, undigested plasmid. Samples not containing spermidine during the digestion received 1 mM spermidine before electrophoresis: lane 7, *Hind*III + 1 mM spermidine; lane 8, *Bam*HI + 1 mM spermidine; lane 9, *Eco*RI + 1 mM spermidine; lane 10, *Pst*I + 1 mM spermidine; lane 11, *Hpa*I + 1 mM spermidine.

3.2. Effect of spermine on endonucleolytic cleavage of plasmid pJDB 207

Fig.2 shows the effect of 0.5 mM spermine on the ability of various restriction enzymes to produce DNA fragments from pJDB 207. The results are similar to those obtained with spermidine: *Hind*III (lane 8) and *Hpa*I (lane 11) were totally inhibited and *Bam*HI (lane 7) and *Pst*I (lane 10) were partially active in the presence of 0.5 mM spermine. With *Eco*RI, bands corresponding to the 6.1, 4.4 and 3.3 kb partial digestion products could be detected, as in the presence of spermidine. With 1 mM spermine (fig.3) and a concentration

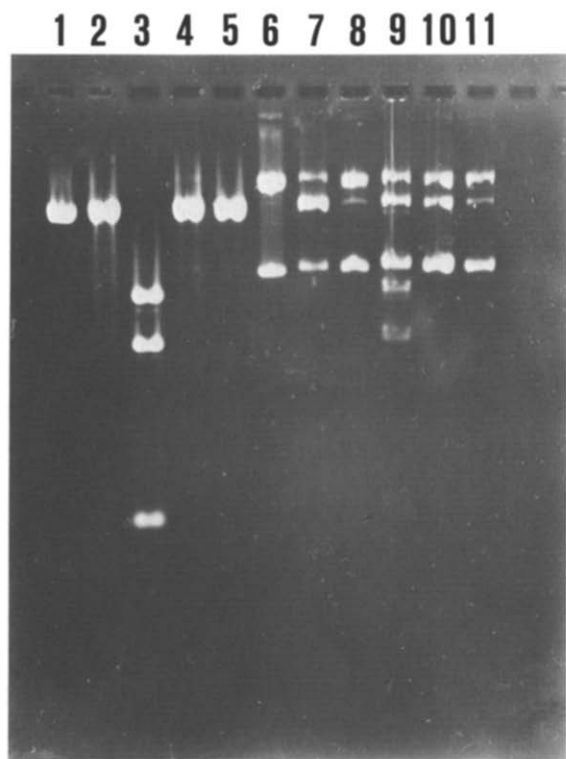


Fig.2. Effect of 0.5 mM spermine on the digestion of pJDB 207 by various restriction endonucleases. Lane 1, *Hind*III; lane 2, *Bam*HI; lane 3, *Eco*RI; lane 4, *Pst*I; lane 5, *Hpa*I; lane 6, undigested plasmid; lane 7, *Bam*HI + 0.5 mM spermine; lane 8, *Hind*III + 0.5 mM spermine; lane 9, *Eco*RI + 0.5 mM spermine; lane 10, *Pst*I + 0.5 mM spermine; lane 11, *Hpa*I + 0.5 mM spermine. Samples not containing spermine during the digestion received 0.5 mM spermine before digestion. Other details as in fig.1.

of *Eco*RI 15 times higher (lane 9) than normally used in these studies (lane 3) the restriction pattern still differed from that observed without polyamines (lanes 2,7), although the 0.8 kb fragment could now be detected. At 2 mM spermine, all the restriction enzymes were completely inhibited (not shown), indicating that spermine is a more powerful inhibitor of endonuclease activity than spermidine.

Putrescine (up to 4 mM) caused no effect whatsoever, suggesting that the carbon chain of putrescine is too short to mask the recognition sites for endonucleases (not shown).

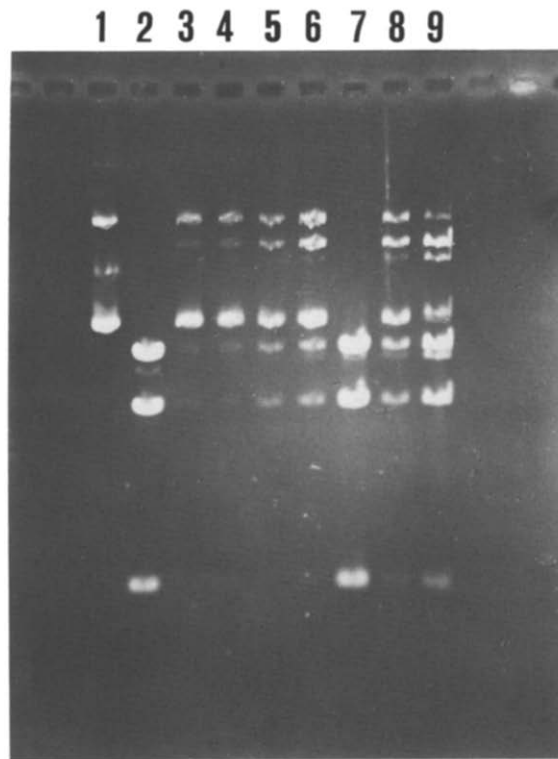


Fig.3. Effect of increasing concentration of *Eco*RI on the cleavage of pJDB 207 in the presence of 1 mM spermine. Lane 1, undigested plasmid; lane 2, plasmid digested with 5 units of *Eco*RI; lane 7, plasmid digested with 5 units of *Eco*RI and then incubated 1 h with 1 mM spermine. In the other lanes, digestion was done in the presence of 1 mM spermine with: lane 3, 5 units; lane 4, 10 units; lane 5, 20 units; lane 6, 30 units; lane 8, 40 units, and lane 9, 75 units of *Eco*RI.

4. DISCUSSION

The mechanism of the inhibition of endonucleases by physiological concentrations of polyamines [1] remains to be determined but the results confirm the general observation that polyamines almost always have an effect on reactions catalyzed by DNA metabolizing enzymes [7-9]. The physiological meaning of the inhibition of endonucleases by polyamines is an open question, although viral DNA has been shown to contain spermidine as an intrinsic component [12]. From the practical point of view, however, the

results clearly show that the common use of polyamines [13] in recombinant DNA chemistry should be done with great caution, because it may cause incomplete digestion in subsequent treatments with restriction enzymes.

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