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SPERMIDINE AND SPERMINE STIMULATE THE ACTIVITY OF T4-DNA LIGASE

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When the ability of T4-DNA ligase from E. coli NM 989 to form higher molecular weight polymers from linearized plasmid pJDB 207 was followed, it was observed that physiological concentrations (0.5 to 1.0 mM) of spermidine and spermine greatly stimulated the formation of these polymers. The effect had a strict specificity since 1,3-diaminopropane, putrescine (1,4-diaminobutane) and N^1-acetylspermidine neither stimulated nor inhibited this activity of DNA ligase. The structural analogues of spermidine, methyl bis(guanylhydrazone) and 1,1'-[(methylethanediylidene)dinitrilo]bis(3-aminoguanidine) totally abolished the stimulatory effect of spermidine on T4-DNA ligase without affecting the enzyme's basal activity.

An increasing body of evidence suggests that polyamines are intimately involved in cell division (1,2). The most obvious explanation for their importance in cell growth is the high affinity of polycationic polyamines towards negatively charged phosphate groups of DNA. X-ray studies (3-5) have provided evidence for the binding of spermidine and spermine to the minor groove of DNA. A recent study has shown by using the ^{23}Na NMR-technique that spermidine and spermine (but not putrescine) are able to neutralize very effectively all phosphate groups in calf thymus DNA (6). This neutralization could be the reason for the fast folding and packing of phage T7 DNA (7,8), which is energetically unfavourable but takes place readily in the presence of spermidine and spermine.

Spermine molecules have been located in tRNA crystals (9,10) and it is well known that polyamines stabilize the conformation of different tRNAs (11,12). Besides the physiochemical evidence of the interaction of polyamines with nucleic acids, it has been reported that polyamines stimulate the activity of DNA gyrase (13,14) and DNA polymerase (15,16). This suggests that polyamines may change the conformation of DNA to form structures more acceptable by enzymes concerned with DNA metabolism.

In this paper we report a marked stimulation of T4-DNA ligase as measured by the ability of the enzyme to form higher molecular-weight adducts of DNA by physiological concentrations of specific polyamines.

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MATERIALS AND METHODS

Chemicals: The physiological polyamines, putrescine, spermidine and spermine (as their hydrochlorides) as well as unphysiological diamines (1,3-diaminopropane and 1,7-diaminoheptane) were from Sigma Co. (St. Louis, MO., U.S.A.). Methyl bis(guanylhydrazone) (MGBG) was from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.). 1,1'-[(methylethanediylidene)dinitrilo] bis(3-aminoguanidine) (MBAG) was synthesized as in (17). N^1-acetylspermidine was a generous gift from Dr. E. Hölttä. Restriction enzymes, T4-DNA ligase and lambda DNAs (for molecular weight markers) were obtained from Boehringer (Mannheim, West-Germany).

Plasmid DNA: Plasmid pJDB 207 (obtained from Dr. Beggs) was used in this study. The plasmid was kept in E. coli JA 221 and to obtain large amounts of plasmid DNA 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 Guerry et al. (18).

T4-DNA ligase reaction: 1-2 μg of the DNA was incubated for 4h at 21° C in 20 μl containing 20 mM Tris/HCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.6 mM ATP, 0.5 milliunits of T4-DNA ligase, and the indicated amounts of polyamines, and the reaction was halted by heating to 65° 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, including molecular weight markers, at a final concentration of 1 mM, immediately before heating, to see any possible effects of polyamines on the migration of DNA during the gel electrophoresis.

Agarose gel electrophoresis: The heated DNA samples were subjected to electrophoresis in 0.5% 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 15-18 h. After the run, DNA bands were visualized by staining with ethidium bromide.

RESULTS AND DISCUSSION

Plasmid pJDB 207 has only one cutting point for restriction enzyme Bam HI (19) and when this plasmid is linearized by Bam HI it yields a 6.9 kb DNA fragment. Fig. 1. shows the effect of increasing concentrations of spermidine on the ability of DNA ligase to form high molecular-weight polymers of this linearized pJDB DNA. With no polyamines present during the ligation (but 1 mM spermidine added before the electrophoresis) the circular monomer was a major product of the ligase reaction, together with dimer and trimer, but no adducts bigger than lambda DNA (49 kb) were formed (lane 4). Spermidine had no significant effect at 0.25 mM, but at 0.5 and 1.0 mM (lanes 6 and 7) no circularized monomer was formed, but several bands at or above 49 kb was formed, and the amour of residual linear monomer decreased, showing that spermidine accelerates the ligation, as well as directing it away from simple circularization of the monomer DNA. Higher concentrations of spermidine (up to 4 mM) did not further change the electrophoretic pattern. The patterns with 4 and 8 mM putrescine (lanes 9 and 10) did not differ significantly from the control whereas 4 mM 1,7-diaminoheptane (lane 8), also did not stimulate the formation of higher polymers of DNA, but possibly prevented the appearance of circularized monomer, although it caused some streaking of the DNA.

The effect of 0.25 to 1.0 mM spermine on the ligase reaction is shown in Fig. 2. Spermine at 0.5 and 1.0 mM (lanes 7 and 8) produced qualitatively the

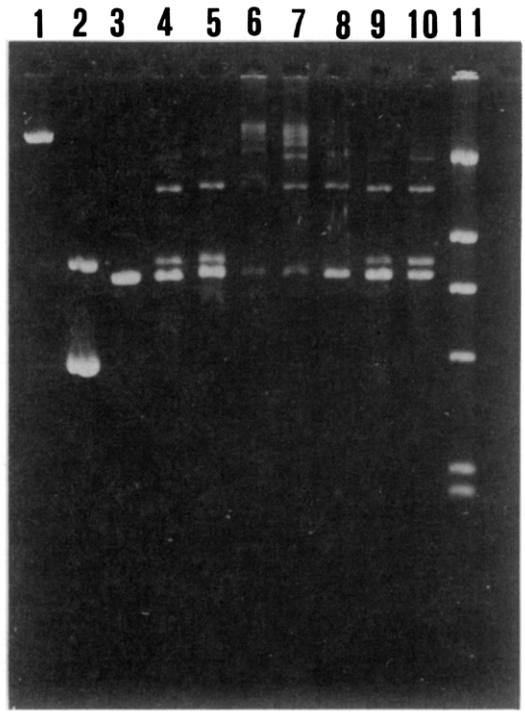

Figure 1: Effect of spermidine, putrescine and 1,7-diaminoheptane on the ligation of DNA.
Plasmid pJDB 207 DNA was linearized with Bam HI and 1.5 μg portions ligated with T4-DNA ligase as described in Materials and Methods with the following additions: lane 4: none (but 1 mM spermidine added before electrophoresis); lanes 5,6 and 7: 0.25, 0.5 and 1.0 mM spermidine, respectively; lane 8: 4 mM 1,7-diaminoheptane; lanes 9 and 10: 4 and 8 mM putrescine, respectively. Also shown are lambda DNA (49 kb, lane 1), undigested plasmid pJDB 207 (lane 2), digested but unligated pJDB 207 (6.9kb, lane 3), and Hind III digested lambda DNA (top to bottom: 23.6, 9.6, 6.6, 4.3, 2.2 and 2,0 kb, (lane 11).

same stimulatory effect as 1 mM spermidine (lane 6). Again 4 mM and 8 mM putrescine (lanes 9 and 10) had no significant effect.

An earlier report (20) has suggested that DNA ligase from calf thymus was stimulated by putrescine as well as spermidine and spermine. In that work, however, the activity of the ligase was measured by following the incorporation of radioactivity from (^{32}P)-ATP into calf thymus DNA, the size of the DNA products was not measured, and the effect of polyamines could be at least partially mimicked by increasing the concentration of Mg^{2+} from 0.5 to 15 mM. In the present work increasing the concentration of Mg^{2+} (up to 30 mM) did not abolish nor mimic the effect of spermidine or spermine on T4-DNA ligase (not shown).

We next wanted to study more closely the specificity of the effect of polyamines on the ligase reaction. Fig. 3 shows the effect on the ligation of plasmid DNA of various compounds used in polyamine chemistry: 1 mM spermidine

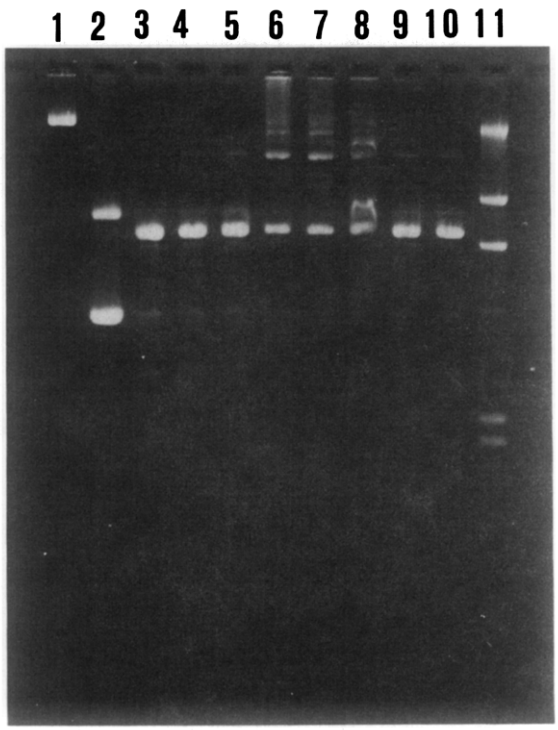

Figure 2: Effect of spermine and putrescine on the ligation of DNA. Ligation assays contained 2 μg of linearized plasmid and the following additions: Lane 4: none (but 1 mM spermine added before electrophoresis); lanes 5,7 and 8: 0.25, 0.5 and 1.0 mM spermine, respectively; lane 6: 1 mM spermidine; lanes 9 and 10: 4 and 8 mM putrescine, respectively. Also shown are lambda DNA (lane 1), undigested plasmid (lane 2), digested un-ligated plasmid (lane 3) and Hind III digested lambda DNA (lane 11).

(lane 5); 10 mM 1,3-diaminopropane (lane 6); 10 mM putrescine (lane 7); and 5 mM N^1-acetylspermidine (lane 10). Spermidine was the only compound causing the formation of high molecular weight DNA components. The stimulatory effect was prevented even only by blocking one amino group (N^1-acetylspermidine).

Fig. 3 also shows the effect of 2 and 4 mM MBAG (lanes 8 and 9) and 2 and 4 mM MGBG (lanes 11 and 12) on the ligase reaction in the presence of 1 mM spermidine (neither MBAG or MGBG had any effect when added alone; not shown). Both drugs totally prevented the stimulation by spermidine but did not interfere with the basal reaction (lane 4). MBAG (21) and MGBG (22) are known to inhibit adenosylmethionine decarboxylase and thus the biosynthesis of polyamines. The present results, however, suggest that MGBG, which is again being used clinically as an antileukemic drug (23), may have a direct effect on the metabolism of DNA competing with physiological polyamines. A possible effect of MGBG on DNA molecules has been observed earlier, since MGBG inhibited the template activity of calf thymus DNA in the DNA polymerase reaction (24).

The reason for the stimulatory effect of specific polyamines on the T4-DNA ligase reaction remains obscure but could be related to the aggregation of

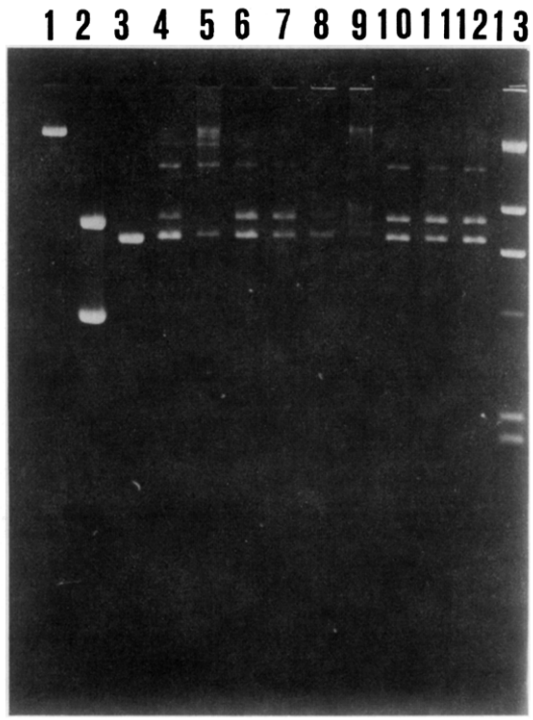

Figure 3: The effect of some other polyamines, MBAG and MGBG on ligation of DNA.
The ligation assays contained 1 μg of linearized plasmid and the following additions: Lane 4: none (but 1 mM spermidine added before electrophoresis); lane 5: 1 mM spermidine; lane 6: 10 mM 1,3-diaminopropane; lane 7: 10 mM putrescine; lanes 8 and 9: 1 mM spermidine + 2 or 4 mM MBAG, respectively; lanes 11 and 12: 1 mM spermidine + 2 or 4 mM MGBG, respectively. Also shown are lambda DNA (lane 1), undigested plasmid (lane 2), digested unligated plasmid (lane 3) and Hind III digested lambda DNA(lane 13).

DNA by spermidine. In an elegant study (25) Krasnow and Cozzarelli studied the effect of spermidine on catenation of DNA by topoisomerases. They showed that spermidine was necessary in this reaction and its effect was due to the compaction of the DNA substrates into aggregates where the high local DNA concentration favored the interlocking of DNA (25). Thus, the aggregation of DNA by polyamines may also play some role in the DNA ligase reaction. It is an attractive possibility that the stimulation of the activity of DNA ligase has some physiological importance, since the polyamine concentrations used in this study are the same as observed in bacteria (1) and in other organisms (2). The stimulation by spermidine or spermine may also have some value in experiments where the maximal activity of T4-DNA ligase is needed such as in blunt end joining, which proceeds much less readily than nick sealing (26,27).

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