# Electron Microscopy and Biochemical Properties of Polyamine-Compacted DNA†

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ABSTRACT: We have obtained polyamine-compacted DNA and analyzed it by electron microscopy employing the method described by Dubochet, suitable for the study of complexes in which the main interactions are of ionic character. In addition, we have developed a simple biochemical method, based on the action of pancreatic DNase I, to demonstrate the condensation of DNA with spermidine. DNA–spermidine complexes are resistant to the action of DNase I, and there is a strong correlation between the presence of condensed DNA forms, both as toroids and as cylinders, and the insensitivity to DNase I activity. We have also shown that pBR322 DNA–spermidine complexes are transcriptionally active in the presence of *Escherichia coli* RNA polymerase. This supports the data concerning the biological activity of spermidine-condensed DNA.

Putrescine, spermidine, and spermine occur ubiquitously in living cells and are present in millimolar concentrations in viruses, bacteria, plants, and actively proliferating animal cells (Ames & Dubin, 1960; Tabor & Tabor, 1984). There is evidence which suggests that polyamines exert a marked effect on many metabolic pathways, in particular those involving nucleic acids, by enhancing the rate of replication, transcription, and translation (Geiger & Morris, 1980; Kurland, 1982; Russell, 1983; Moussatche, 1985). Interruption of polyamine synthesis by mutations (Hafner et al., 1979) or inhibitors (Pegg, 1986) results in reduction or absence of cell growth.

The condensation of DNA into compact structures is a common feature of native genomes. In bacteria, DNA is packed by non-histone proteins, polyamines, and RNA (Flink & Pettijhon, 1975). Viral genomes are compacted by internal viral proteins and polyamines (Ames & Dubin, 1960; Laemmli, 1975). In eukaryotic cells, DNA is compacted into chromatin fundamentally by histones and non-histone proteins. However, the role of polyamines in the higher order condensation of chromatin is practically unknown, as well as their effect on compacted DNA expression. It has been shown that this DNA has biological activity; SV40 DNA is transcribed in vivo as a nucleoprotein complex tightly compacted by histones, that is, as a minichromosome (Gariglio et al., 1979).

Under certain conditions, the in vitro interaction of DNA with polycations (including polyamines) or neutral polymers results in a phase transition from the extended DNA conformation to compact structures (toroids). In general, DNA condensation has been studied by physical methods (Gosule & Schellman, 1978; Tabor & Tabor, 1984; Schellman & Parthasarathy, 1984). Several laboratories have studied DNA organization in polyamine-condensed systems using biochemical approaches with micrococcal nuclease, restriction endonucleases, and DNA gyrase (Marx & Reynolds, 1982; Krasnow & Cozzarelli, 1982; Pingoud et al., 1984).

In this study, employing electron microscopy and a simple biochemical method based on the action of pancreatic DNase I, we have found a strong correlation between the presence of condensed DNA forms, both toroids and cylinders, and their insensitivity to DNase I. We also demonstrate that spermidine-condensed DNA forms allow (with *Escherichia coli* DNA-dependent RNA polymerase) higher transcriptional activity than naked DNA.

## Materials and Methods

*Preparation of DNA.* The pBR322 plasmid DNA was obtained from *Escherichia coli* J54 by gentle phenol extractions (Birnboinm & Doly, 1979). Plasmid DNA dissolved in 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 10 mM ethylenediaminetetraacetic acid (EDTA), and 500 mM NaCl, pH 8, was fractionated in a Sepharose 4B column. The 260/280-nm ratio of plasmid DNA was 2.0. Approximately 70% of the DNA was in the form of supercoiled molecules as judged from agarose gel electrophoresis.

*DNA Concentration.* DNA concentration was determined by Burton's method (Burton, 1956) and by titration against a standard on an ethidium bromide–agarose plate (Maniatis et al., 1982).

*Preparation of DNA–Spermidine Complexes.* Solutions of DNA and chromatographically pure spermidine ($R_f$ = 0.28) were prepared in 10 mM Tris-HCl and 1 mM NaCl, pH 7, mixed, and allowed to stand at 5 °C for 12 h. The final DNA concentration was 33 μg/mL while spermidine concentrations were between 0.57 and 16.5 mM.

*Electron Microscopy.* To analyze DNA–spermidine complexes, we used Dubochet's method; this method has been successfully employed to observe SV40 minichromosomes (Gariglio et al., 1979); 200-mesh copper grids previously shadowed with carbon were ionized with pentylamine; 20-μL samples were placed on freshly prepared grids, and DNA–spermidine complexes were allowed to adsorb for 2 min. Grids were stained with a 2% aqueous solution of uranyl acetate and then shadowed with Pt–Pd (80–20%) for 6 s at $5 \times 10^{-6}$ T, 32–35 A, with an 8° inclination angle.

Naked DNA was observed by using the method described by Kleinschmidt (1968). The hyperphase (50 μL) contained

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1 μg of pBR322 DNA, 5 μg of cytochrome *c*, and 50% distillated formamide in 100 mM Tris-HCl–10 mM EDTA, pH 8.5; the hypophase contained 16% distillated formamide in 10 mM Tris-HCl–1 mM EDTA, pH 8.5. Grids were covered with parlodium film and with a protein–nucleic acid monolayer, stained with a 0.01 mM aqueous solution of uranyl acetate, treated with isopentane, and drained. Grids were shadowed as above, with a 6° inclination angle. A JEOL JEM-100SX transmission electron microscope was used at 60-kV accelerating voltage for all studies.

*Cleavage of DNA–Spermidine Complexes by Pancreatic DNase I.* Ten-microliter samples of DNA–spermidine complexes containing 2 μg of DNA and 0.57–16.5 mM spermidine were incubated with $2.5 \times 10^{-3}$ Kunitz unit of pancreatic DNase I in 10 mM Tris-HCl, 1 mM NaCl, and 1 mM $MnCl_2$, pH 7, at 37 °C for various times. Different aliquots were used for each time period. Reaction was stopped by adding 5 μL of a solution containing 10 mM EDTA, 10 mM urea, 0.05% xylene cyanol, and 0.05% bromophenol blue. Reaction products were analyzed by electrophoresis in 2% agarose gels. Gels were stained with ethidium bromide and photographed by using a Polaroid MP-4 camera and a CHROMATOVUE transilluminator. Quantitative results were obtained by analyzing the gel negatives with a scanner (Gelman DCD 16). The scans were digitized and numerically integrated; the relative amount of the cleavage products in each sample was normalized to the original DNA concentration. As a control, 2 μg (10 μL) of pBR322 DNA was incubated with $2.5 \times 10^{-3}$ Kunitz unit of DNase I, and the products were analyzed by electrophoresis in 2% agarose gels, as indicated above.

*Transcription of DNA–Spermidine Complexes.* RNA polymerase was prepared from *E. coli* SA1943 according to Burgess and Jendrisak (1975) and contains a full subunit component as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The DNA was associated (12 h at 5 °C) to 0, 0.57, 1.15, or 4.7 mM spermidine. The transcription assays were carried out in a final volume of 300 μL, which contained 12 μg of pBR322 DNA (or DNA–spermidine complexes, see above), 10 mM Tris-HCl, pH 7.9, 4 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol, 0.5 mM each of ATP, CTP, and GTP, 70 μM [$^3$H]UTP (specific activity 285 cpm/pmol), and 30 μg of DNA-dependent RNA polymerase from *E. coli*. The reaction was carried out at 37 °C for the indicated times (see Figure 5). Aliquots were removed for [$^3$H]RNA determination (60 μL), electron microscopy (10 μL), and DNase I treatment (10 μL); the [$^3$H]UMP incorporation was determined basically as previously described (Gariglio et al., 1979).

## Results

In this study, we have applied three different approaches to characterize DNA–spermidine complexes: electron microscopy (a method that has been employed to show DNA condensation), a biochemical approach based on the endonuclease activity of DNase I, and a biological activity determined by in vitro transcription.

*Electron Microscopy of Condensed pBR322 DNA.* Electron microscopy by Dubochet's method shows that spermidine concentration below 1.15 mM is not efficient to compact DNA, since only extended pBR322 molecules were observed (Figure 1a). At a spermidine concentration of 1.15 mM or higher, approximately half of the pBR322 DNA was condensed in compact structures similar to the toroids described by Gosule and Schellman (1978) (Figure 1c,d), and the remaining fraction was observed as large cylindric aggregates, similar to those described by Allison et al. (1981) (Figure 1b,f,g). We have not observed in any case extended fibrous structures simultaneously with toroidal and cylindrical forms, suggesting that all the DNA molecules were condensed with spermidine. As the spermidine concentration increased, the size of the hole of toroids became smaller, and toroids were thicker and with clearer boundaries (compare panels c and d of Figure 1); furthermore, the large aggregates (cylindric forms) tended to be shorter with clear boundaries and without branching off (compare panels b and f of Figure 1). End loops were observed in some cylindric forms (Figure 1g), suggesting that DNA is folded as proposed by Eickbush and Moudrianakis (1978). Figure 1j shows a low magnification overview of a typical preparation of pBR322 DNA–spermidine complexes with 16.5 mM spermidine. When 116 mM NaCl was added to the pBR322 DNA–spermidine complexes and these were incubated at room temperature, electron microscopy showed no compact structures; only extended forms identifiable as DNA molecules could be observed (Figure 1h), suggesting that DNA compaction can be reversed at this salt concentration, even in DNA–spermidine complexes prepared with spermidine concentrations as high as 16.5 mM. Electron microscopy by Dubochet's method shows that when spermidine was excluded, keeping other steps in the specimen preparation unaltered, only extended pBR322 DNA molecules were obtained (Figure 1i). Electron microscopy by Kleinschmitd's method also shows the classic image of naked pBR322 DNA (Figure 1k).

Electron microscopy of calf thymus DNA–spermidine complexes show toroids bigger than pBR322 DNA complexes, probably due to the greater size of calf thymus DNA segments utilized (see Figure 1d,e).

*Resistance of DNA–Spermidine Complexes to Pancreatic DNase I.* Spermidine and spermine turn plasmid DNA resistant to restriction endonuclease cleavage (Pingoud et al., 1984; Kuosmanen & Poso, 1985). The inhibitory effects that polyamines have on the cleavage of DNA by restriction endonucleases may be explained by small conformational changes of the substrate or by a decreased accessibility of the substrate for the enzyme (Pingoud et al., 1984); trying to detect bigger conformational changes in DNA, we decided to find out whether DNA–spermidine complexes were also resistant to the action of pancreatic DNase I, a broader hydrolytic enzyme.

pBR322 DNA was digested by DNase I at concentrations of spermidine below 1.15 mM (Figure 2), when DNA is probably associated to spermidine but not compacted (see Figure 1a). At spermidine concentrations of 1.15 mM or higher, when the association of this polyamine with pBR322 DNA forms compact structures as shown by electron microscopy (see Figure 1b–d,f), this DNA remained practically undigested by DNase I. Control (c) was rapidly digested.

To discard a possible spermidine inhibitory effect on DNase I activity, we followed DNase I activity on pBR322 DNA–spermidine complexes previously dissociated with 116 mM NaCl. Figure 3 shows the action of DNase I on pBR322 DNA–spermidine complexes containing 16.5 mM spermidine (at these spermidine concentrations, the highest condensation was observed by electron microscopy; Figure 1d,g). These complexes were incubated for different times with the endonuclease. It can be seen that in DNA–spermidine complexes the DNA remained practically undigested by DNase I for any length of time up to 60 min (lanes e). On the other hand, naked pBR322 DNA was partially digested after 5 min and was totally digested after 30 min (lanes c). No inhibition of the enzyme by spermidine was observed in the digestion of DNA dissociated from the DNA–spermidine complex with 116 mM NaCl (lanes f). In this case, spermidine was present

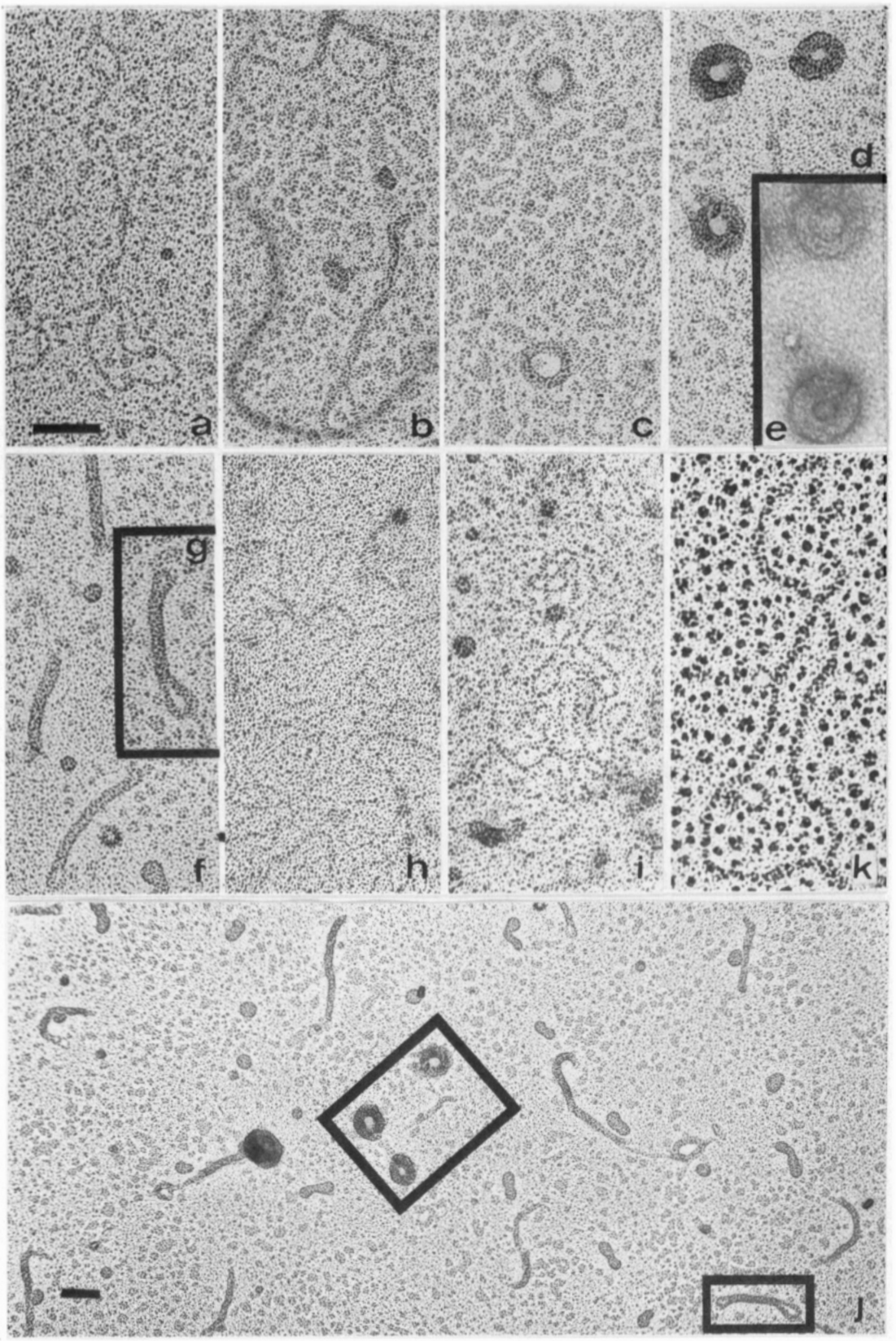


FIGURE 1: Electron micrography of DNA–spermidine complexes. All preparations were contrasted with uranyl acetate and shadowed linearly with Pt–Pd by Dubochet's method (Dubochet et al., 1971). pBR322 DNA–spermidine complexes with 33 $\mu$g/mL DNA and (a) 0.57 mM, (b and c) 1.15 mM, and (d, f, and g) 16.5 mM spermidine; (h) complexes as in (d) and (f) but dissociated for 5 min with 116 mM NaCl; (e) calf thymus DNA–spermidine complexes with 33 $\mu$g/mL DNA and 16.5 mM spermidine; (i) pBR322 DNA without spermidine; (j) a low magnification overview including toroids and cylinders of (d) and (g); (k) pBR322 DNA by Kleinschmidt's method (Kleinschmidt, 1968). Solid lines represent 0.1 $\mu$m. Rectangles indicated toroids and cylinders that were magnified in (d) and (g).

in the incubation system, but it is not condensing pBR322 DNA, due to the high salt concentration, as demonstrated by electron microscopy (Figure 1h); the presence of spermidine in the dissociated form appears to stimulate the activity of DNase I, since pBR322 DNA dissociated from spermidine by 116 mM NaCl was rapidly digested by the enzyme after only 15 min of incubation (see lanes f). Figure 3 shows that 116 mM NaCl caused a slight DNase I inhibition; pBR322 DNA

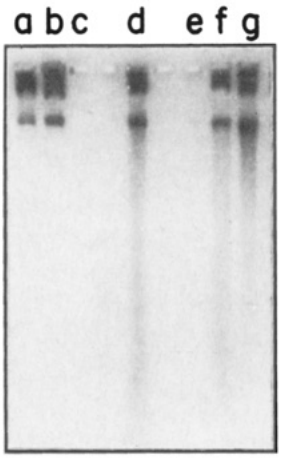


FIGURE 2: Effect of spermidine on DNA cleavage by pancreatic DNase I. pBR322 DNA–spermidine complexes were prepared with 33 $\mu$g/mL DNA, as mentioned under Materials and Methods. (Lane a) 2 $\mu$g of pBR322 and (lane b) complexes with 2 $\mu$g of pBR322 DNA and 16.5 mM spermidine are controls without enzyme. Lanes c–g are 10-$\mu$L samples in 10 mM Tris-HCl, 1 mM NaCl, and 1 mM $MnCl_2$, pH 7, incubated 30 min at 37 °C with $2.5 \times 10^{-3}$ Kunitz unit of DNase I: (c) 2 $\mu$g of pBR322; (d) complexes with 2 $\mu$g of pBR322 DNA and 16.5 mM spermidine; (e–g) pBR322 DNA–spermidine complexes containing 2 $\mu$g of DNA and 0.57, 1.15, and 4.7 mM spermidine, respectively.

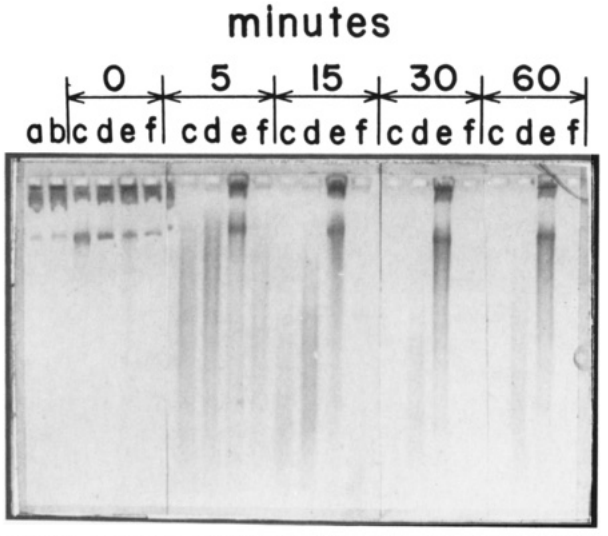


FIGURE 3: Resistance of pBR322 DNA–spermidine complexes to pancreatic DNase I. Reactions and DNA concentrations are as indicated in Figure 2. (Lane a) 2 $\mu$g of pBR322 and (lane b) complexes with 2 $\mu$g of pBR322 DNA and 16.5 mM spermidine are controls without enzyme. Lanes c–f are 10-$\mu$L samples incubated with DNase I at indicated times: (c) 2 $\mu$g of pBR322; (d) 2 $\mu$g of pBR322 + 116 mM NaCl; (e) complexes with 2 $\mu$g of pBR322 DNA and 16.5 mM spermidine; (f) complexes as in (e) but dissociated for 30 min with 116 mM NaCl before addition of DNase I.

at this salt concentration was partially digested after 60 min of incubation with the enzyme (see lanes d); however, the inhibitory effect of NaCl on DNase I was overcome by the stimulating effect of spermidine, as demonstrated by the total degradation of DNA in the presence of 116 mM NaCl and 16.5 mM spermidine (see lanes f). The cleavage reaction kinetics of plasmid DNA by pancreatic DNase I are shown in Figure 4. From this quantitative evaluation, it is apparent that only the DNA of pBR322 DNA–spermidine complexes was not sensible to DNase I up to 20 min; this lack of sensibility can probably be explained as a result of the high degree of DNA compaction observed by electron microscopy. It can be seen that spermidine does not have any inhibitory effect on DNase I, but rather the opposite; the rate of DNA cleavage

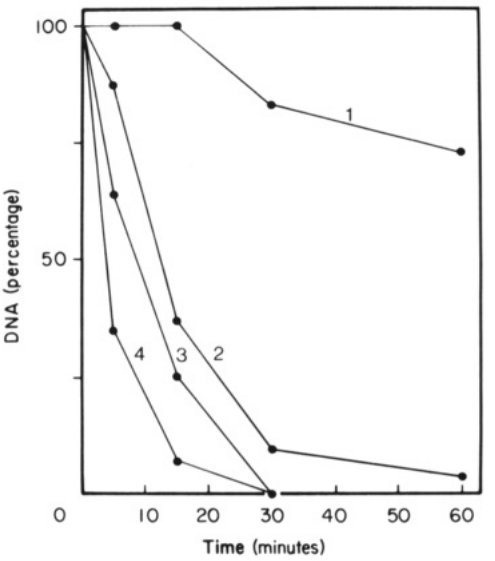


FIGURE 4: Quantitative evaluation of pancreatic DNase I kinetics. Reactions described in Figure 3 (incubated with DNase I) were evaluated with a gel scanner; DNA percent was calculated as indicated under Materials and Methods. (1) pBR322 DNA–spermidine complexes (2 $\mu$g of DNA and 16.5 mM spermidine); (2) 2 $\mu$g of pBR322 DNA + 116 mM NaCl; (3) 2 $\mu$g of pBR322 DNA; (4) complexes as in (1), but dissociated for 30 min with 116 mM NaCl before addition of DNAse I.

is enhanced when 16.5 mM spermidine is present but not associated to DNA due to a high salt concentration (see Figure 4, curve 4).

The possibility that spermidine binds to DNase I and changes its cleavage specificity was also ruled out as follows. When DNase I was exposed for 12 h to 16.5 mM spermidine in 10 mM Tris-HCl–1 mM NaCl, pH 7, at 4 °C and dialyzed against the same buffer, the enzyme activity was as shown in Figure 3c,d; i.e., plasmid DNA was completely digested, and DNA–spermidine complexes were not digested. These results suggested that spermidine does not bind to DNase I. This procedure has been used before to rule out the possibility that polyamines bind to nucleases (Shishido, 1985).

*In Vitro Transcriptional Activity of pBR322 DNA–Spermidine Complexes.* In vitro, polyamines stimulate transcription by bacterial and eukaryotic polymerases (Russell, 1983; Tabor & Tabor, 1984; Moussatche, 1985); however, in these experiments, polyamine DNA condensation was not considered. For this reason, we decided to study the effect of condensing and noncondensing polyamine concentrations on in vitro transcription. Most transcriptional experiments were at low ionic strength (10 mM Tris-HCl–10 mM KCl) in order to be sure that pBR322 DNA–spermidine complexes remain in their compact form. Figure 5 shows the activity of *E. coli* DNA-dependent RNA polymerase on pBR322 DNA–spermidine complexes. It can be seen that under conditions in which no compact structures were observed by electron microscopy (pBR322 DNA–spermidine complexes with 0.57 mM spermidine), RNA synthesis was practically the same as with naked pBR322 DNA. When we used DNA–spermidine complexes with 1.15 mM spermidine, the lowest spermidine concentration that caused DNA condensation (see Figure 1b,c), about a 90-fold increase in transcription was observed. DNA–spermidine complexes obtained with 4.7 mM spermidine, which form more compacted structures, cause almost a 4-fold increase in transcription. Parallel electron microscopy studies of DNA–spermidine complexes obtained with 1.15 and 4.7 mM spermidine showed the presence of toroids during transcription. These complexes were also resistant to the

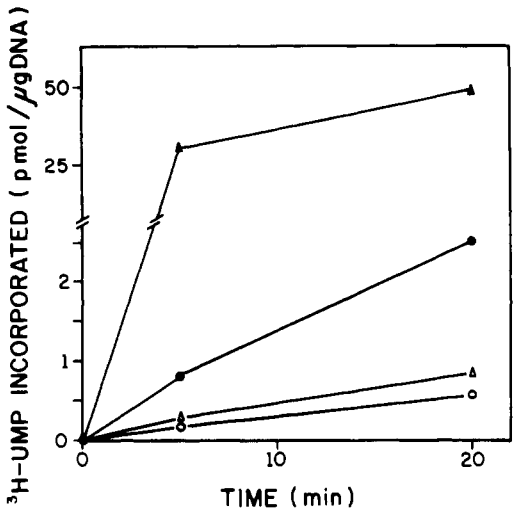


FIGURE 5: Transcription kinetics of DNA–spermidine complexes. pBR322 DNA–spermidine complexes were preformed and incubated for RNA synthesis (37 °C) as described under Materials and Methods. At the indicated times, parallel reactions were carried out with pBR322 DNA previously associated with 0.57 (△), 1.15 (▲), or 4.7 mM (●) spermidine. As a control, a reaction was executed in the absence of spermidine (○).

hydrolytic action of DNase I (not shown).

When transcription was carried out at higher ionic strength (50 mM Tris-HCl–50 mM KCl), only a 2-fold transcription increase was observed with DNA–spermidine complexes containing 1.15 mM spermidine (not shown). At this ionic strength, we found that spermidine–DNA complexes were dissociated, as demonstrated by DNase I activity.

## Discussion

We employed Dubochet's method for electron microscopy studies of pBR322 DNA–spermidine complexes since the interactions between DNA and polyamine include electrostatic interactions (Manning, 1980; Schellman & Parthasaraty, 1984), as in SV40 minichromosomes (Gariglio et al., 1979). On the other hand, in this method the grids are not treated with poly(L-lysine) (Widmon & Baldwin, 1980) or cytochrome (Chattoraj et al., 1978), which can influence not only the apparent diameter of DNA toroids (Widmon & Baldwin, 1980) but also the affinity of spermidine to DNA. Using this method, we have observed toroids and cylindric forms similar to those previously described (Gosule & Schellman, 1978; Allison et al., 1981); however, spheroids were never seen. Furthermore, no irregular forms, suggesting precipitation of DNA by spermidine, were detected, indicating that it is possible to use DNA concentrations of 33 $\mu$g/mL to obtain compact DNA without nucleic acid losses. At the ionic strength used in this work (10 mM Tris-HCl–1 mM NaCl, pH 7), the minimum polyamine concentration that successfully compacts DNA was 1.15 mM. This value is very close to the one reported in the phase diagram for DNA condensation by Krasnow and Cozzarelli (1982). As spermidine concentration increases, electrostatic interactions between DNA and spermidine also rise, resulting in an increase in toroid width while cylindrical forms tend to decrease in size. End loop in some cylinders suggests that every deposition of DNA on the rod must be accompanied by exposition of a subsequent DNA segment in the opposite direction, as proposed by Eickbush and Moudrianakis (1978).

It is known that the activity of some DNA-metabolizing enzymes is affected by spermidine (Tabor & Tabor, 1984). It is important to distinguish if this effect is due to DNA compaction, to competition between cationic domains of the enzyme and spermidine in binding to DNA, or to direct inhibition of the enzyme activity. Our results suggest that DNase I inactivity on DNA–spermidine complexes (either pBR322 or calf thymus DNA) is due to compaction of DNA by spermidine. When we dissociate DNA–spermidine complexes by increasing the ionic strength, free spermidine does not show any inhibitory effect on the enzyme; on the contrary, it has a stimulating effect, suggesting that spermidine does not compete with DNase I for binding to DNA and does not inhibit the enzyme. At present, we cannot explain the stimulating effect of spermidine on DNase I activity.

DNase I is a broader hydrolytic enzyme than restriction endonucleases; thus, the inhibitory effect that polyamine has on DNA cleavage by DNase I may be explained by bigger conformational changes in DNA–polyamine complexes than those postulated for restriction enzymes. Furthermore, we found a correlation between enzymatic and structural conformational changes of DNA–spermidine complexes. Our results with DNase I represent a simple, rapid, and reproducible biochemical method that allows the detection of DNA–spermidine condensation.

It is important to note that DNA compaction by spermidine does not convert DNA into an inert molecule. Krasnow and Cozzarelli (1982) have suggested that polyamine should facilitate any reaction which requires the interaction of DNA segments, such as DNA ring catenation by DNA topoisomerases, as well as those which involve compaction phenomena such as packaging of DNA into phage particles. Besides, these authors have estimated that the complex sequence of reactions of $\phi$X174 DNA replication (Schekman et al., 1974) takes place efficiently in spermidine concentrations that probably induce DNA compaction. In this work, we found that condensed pBR322 DNA–spermidine complexes presented a greater transcriptional activity than naked DNA molecules. We observed that the maximum increase in transcription (about 90-fold) was with DNA–spermidine complexes formed in 1.15 mM spermidine, suggesting that some order in the tridimensional structure of DNA is required for maximum transcriptional activity. Direct spermidine stimulation on RNA polymerase activity is discarded, because at higher ionic strength (in which DNA is not compacted by spermidine) only a 2-fold increase in transcription was observed. Besides, in the presence of spermidine concentrations (0.57 mM) that are not condensing DNA, the effect on transcription is minimal. It was also observed that pBR322 remains condensed during transcription, supporting the biological accessibility of DNA in DNA–spermidine-condensed structures. It has been reported that polyamines induce a structural B to Z transition of DNA (Thomas & Bloomfield, 1984), which could be related to transcriptional enhancement (Nordheim & Rich, 1984). We are currently trying to investigate the molecular mechanism of the increased transcriptional activity of compacted DNA–spermidine complexes.

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**Registry No.** DNase, 9003-98-9; RNA polymerase, 9014-24-8.

## References

Allison, S. A., Herr, J. C., & Schurr, M. J. (1981) *Biopolymers 20*, 469–488.

Ames, B. N., & Dubin, D. T. (1960) *J. Biol. Chem. 253*, 769–775.
Birnboinm, H. C., & Doly, J. (1979) *Nucleic Acids Res. 7*, 1513–1518.
Burgess, R. R., & Jendrisak, J. J. (1975) *Biochemistry 14*, 4634–4638.
Burton, K. (1956) *Biochem. J. 62*, 315–324.
Chattoraj, D. K., Gosule, L. C., & Schellman, J. A. (1978) *J. Mol. Biol. 121*, 327–337.
Dubochet, J., Ducommun, M., Zollinger, M., & Kellenberg, E. (1971) *J. Ultrastruct. Res. 35*, 147–167.
Eickbush, T. H., & Moudrianakis, E. N. (1978) *Cell (Cambridge, Mass.) 13*, 295–306.
Flink, I., & Pettijhon, D. E. (1975) *Nature (London) 253*, 62–64.
Gariglio, P., Llopis, R., Oudet, P., & Chambon, P. (1979) *J. Mol. Biol. 131*, 75–105.
Geiger, L. E., & Morris, D. R. (1980) *J. Bacteriol. 141*, 1192–1198.
Gosule, L. C., & Schellman, J. A. (1978) *J. Mol. Biol. 121*, 311–326.
Hafner, E. W., Tabor, C. W., & Tabor, H. (1979) *J. Biol. Chem. 254*, 12419–12426.
Kleinschmidt, A. K. (1968) *Methods Enzymol. 12*, 361–377.
Krasnow, M. A., & Cozzarelli, N. R. (1982) *J. Biol. Chem. 257*, 2687–2693.
Kuosmanen, M., & Poso, H. (1985) *FEBS Lett. 179*, 17–20.
Kurland, C. G. (1982) *Cell (Cambridge, Mass.) 28*, 201–203.
Laemmli, U. K. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4288–4292.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, p 468, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Manning, G. S. (1980) *Biopolymers 19*, 37–59.
Marx, K. A., & Reynolds, T. C. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6484–6488.
Moussatche, N. (1985) *Biochim. Biophys. Acta 826*, 113–120.
Nordheim, A., & Rich, A. (1983) *Nature (London) 303*, 674–678.
Pegg, A. E. (1986) *Biochem. J. 234*, 249–262.
Pingoud, A., Urbanke, C., Alves, J., Ehbrecht, H. J., Zabeau, M., & Gualerzi, C. (1984) *Biochemistry 23*, 5697–5703.
Russell, D. H. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1318–1321.
Schekman, R., Weiner, A., & Kornberg, A. (1974) *Science (Washington, D.C.) 186*, 987–993.
Schellman, J. A., & Parthasarathy, N. (1984) *J. Mol. Biol. 175*, 313–329.
Shishido, K. (1985) *Biochim. Biophys. Acta 826*, 147–150.
Tabor, C. W., & Tabor, H. (1984) *Annu. Rev. Biochem. 53*, 749–790.
Thomas, T. J., & Bloomfield, U. A. (1984) *Biopolymers 23*, 1295–1306.
Widmon, J., & Baldwin, R. L. (1980) *J. Mol. Biol. 144*, 431–453.

# Polycyclic Aromatic Hydrocarbons Physically Intercalate into Duplex Regions of Denatured DNA†

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ABSTRACT: We have investigated the physical binding of pyrene and benzo[*a*]pyrene derivatives to denatured DNA. These compounds exhibit a red shift in their absorbance spectra of 9 nm when bound to denatured calf thymus DNA, compared to a shift of 10 nm when binding occurs to native DNA. Fluorescence from the hydrocarbons is severely quenched when bound to both native and denatured DNA. Increasing sodium ion concentration decreases binding of neutral polycyclic aromatic hydrocarbons to native DNA and increases binding to denatured DNA. The direct relationship between binding to denatured DNA and salt concentration appears to be a general property of neutral polycyclic aromatic hydrocarbons. Absorption measurements at 260 nm were used to determine the duplex content of denatured DNA. When calculated on the basis of duplex binding sites, equilibrium constants for binding of 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene to denatured DNA are an order of magnitude larger than for binding to native DNA. The effect of salt on the binding constant was used to calculate the sodium ion release per bound ligand, which was 0.36 for both native and denatured DNA. Increasing salt concentration increases the duplex content of denatured DNA, and it appears that physical binding of polycyclic aromatic hydrocarbons consists of intercalation into these sites.

Polycyclic aromatic hydrocarbons (PAH)[1] are an important class of biologically active compounds. They are mutagenic and carcinogenic and undergo physical and covalent interactions with DNA (Heidelberger, 1975; LeBreton, 1985). Although numerous reports on the physical intercalation of charged aromatic dyes have appeared (Berman & Young, 1981; Dougherty & Pilbrow, 1984), much less attention has been given to the weaker binding of neutral PAH to DNA.

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[1] Abbreviations: PAH, polycyclic aromatic hydrocarbons; BP, benzo[*a*]pyrene; *trans*-tetrol, racemic 7*r*,8*t*,9*t*,10*c*-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; *cis*-tetrol, racemic 7*r*,8*t*,9*t*,10*t*-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; 7,8-di(OH)$H_2$BP, racemic *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene; 7,8-di(OH)$H_4$BP, racemic *trans*-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BPDE, (±)-7*r*,8*t*-dihydroxy-9*t*,10*t*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; EDTA, ethylenediaminetetraacetic acid; DMSO, dimethyl sulfoxide.

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