Effects of Polyamines and Methylglyoxal Bis(guanylhyazone) on Hepatic Nuclear Structure and Deoxyribonucleic Acid Template Activity

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1. The interaction of polyamines and methylglyoxal bis(guanylhyazone) {1,1'-[(methylethanediyldene)-dinitrilo]diguanidine} with isolated rat liver nuclei was investigated by electron microscopy. 2. At 4mM, putrescine was without effect; however, spermidine, spermine or methylglyoxal bis(guanylhyazone) resulted in dispersed chromatin and alterations in nucleolar structure. In addition, spermidine or methylglyoxal bis(guanylhyazone) caused marked aggregation of interchromatin granules. 3. The DNA template property of calf thymus DNA was examined by using DNA polymerases from *Escherichia coli*, *Micrococcus lysodeikticus* and calf thymus in the presence of 0–5mM-amine. 4. In the presence of DNA polymerase, spermine or methylglyoxal bis(guanylhyazone) inhibited activity, whereas putrescine or spermidine had much less effect or in some cases stimulated [³H]dTMP incorporation. 5. Template activity which was inhibited by spermine or methylglyoxal bis(guanylhyazone) could be partially restored by additional DNA or enzyme. 6. When mixed with calf thymus DNA, calf thymus histone inhibited template activity as measured with *E. coli* DNA polymerase. The template activity of such a 'histone–nucleate' could not be restored by putrescine, spermidine, spermine or methylglyoxal bis(guanylhyazone). 7. DNA template activity of isolated rat liver nuclei was tested by using *E. coli* DNA polymerase. None of the amines was able to increase the template activity of the nuclear DNA *in vitro*.

Recently there has been great interest in the occurrence and metabolism of polyamines. Much of this interest has been directed toward the possible relationship of polyamines to the growth and development of organisms or tissues (for reviews see Stevens, 1970; Cohen, 1971; Bachrach, 1973; Russell, 1973). Of prime importance are the findings of increased polyamine concentrations in actively proliferating tissues such as regenerating rat liver (Snyder & Russell, 1970), androgen-stimulated prostate glands (Pegg *et al.*, 1970), animal tumours (Marton & Heby, 1974) and in patients with neoplastic diseases (Russell, 1971; Russell *et al.*, 1971; Marton *et al.*, 1974).

During the past few years our laboratory has studied the effects of anionic polyelectrolytes on the DNA template activity of purified DNA or isolated cell nuclei in the presence of DNA polymerase (Brown & Coffey, 1971*a,b*, 1972, 1973; Arnold *et al.*, 1972). In the course of these studies we have now examined the effects of the polyamines and methylglyoxal bis(guanylhyazone) {1,1'-[(methylethanediyldene)-dinitrilo]diguanidine} on the DNA template activity of purified calf thymus DNA and of isolated rat liver cell nuclei.

The polyamines are small nitrogenous bases and commonly include putrescine, spermidine and spermine. Methylglyoxal bis(guanylhyazone), an analogue of the polyamines, is a potent anti-

neoplastic agent (Mihich, 1963, 1965) and appears to be a specific inhibitor of *S*-adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972), an enzyme in the synthetic pathway of both spermidine and spermine. Since these molecules carry a net positive charge at neutral pH we wished to determine if they could interact with naked DNA or DNA–protein complexes in such a way as to alter the DNA template properties toward exogenous DNA polymerase.

In our experiments when naked DNA was used as the template, putrescine or spermidine had little inhibitory effect and sometimes stimulated activity, whereas spermine or methylglyoxal bis(guanylhyazone) inhibited DNA polymerase activity. When a DNA–histone complex (histone–nucleate) was used as the template source, none of the amines stimulated template activity. In addition, although the polyamines altered the structure of isolated hepatic cell nuclei, they had no effect on the ability of DNA, in isolated nuclei, to serve as a template for DNA polymerase.

Experimental

Materials

Male Sprague–Dawley rats (250g) were purchased from National Lab Animal, St. Louis, Mo., U.S.A.

Escherichia coli DNA polymerase (deoxyribonucleoside triphosphate-DNA deoxynucleotidyl-transferase, EC 2.7.7.7) (fraction VII, 5000 units/mg) was purchased from General Biochemicals, Chagrin Falls, Ohio, U.S.A. *E. coli* DNA polymerase (EC 2.7.7.7) (fraction IV; 150 units/mg) was purchased from P-L Biochemicals, Milwaukee, Wis., U.S.A. *Micrococcus lysodeikticus* DNA polymerase, (100 units/mg) was obtained from Miles Laboratories, Elkhart, Ind., U.S.A. One unit of prokaryotic DNA polymerase catalyses the incorporation of 10 nmol of total nucleotide/30 min with poly(dAT) as the primer. Calf thymus DNA polymerase (replicative deoxynucleotidyl-transferase; 100 units/mg) was purchased from General Biochemicals. One unit of calf thymus DNA polymerase catalyses the incorporation of 4 nmol of total nucleotide/h with DNA as the primer. All other components of the DNA polymerase assay system, the polyamines, and the unfractionated histones were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Methylglyoxal bis(guanyldiazotane) was purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A.

Methods

Preparation of heat-denatured DNA. Purified calf thymus DNA (Sigma) was dissolved slowly in $0.1 \times$ SSC (0.015 M-NaCl - $0.0015 \text{ M-sodium citrate}$) by standing at 4°C , with occasional swirling, for 48–72 h. The solution, containing approx. 1 mg of DNA/ml, was centrifuged at 20000g for 10 min. The supernatant was decanted and analysed spectrophotometrically to determine the DNA concentration [$E_{1\%}$ at $260 \text{ nm} = 202$ (Ceriotti, 1952)]. Heat-denatured DNA was prepared by placing DNA, as prepared above, in a boiling-water bath for 10 min, then plunging it into an ice-water bath.

Preparation of isolated nuclei. Animals were killed by cervical fracture. Their livers were rapidly excised and placed in iced vessels. All subsequent steps in the preparation were performed at 0 – 4°C . Rat liver nuclei were prepared by the method of Blobel & Potter (1966). The isolated nuclei were washed twice in buffer A [50 mM-Tris-HCl ($\text{pH } 7.5$ at 25°C)- 5 mM-MgCl_2 - 25 mM-KCl] by resuspension with a Dounce homogenizer (A pestle) and centrifugation at 1000g for 5 min. The isolated washed nuclei were resuspended in buffer A at a final concentration of about 1 mg of DNA/ml as measured by the diphenylamine reaction. Nuclear preparations were kept at 0 – 4°C and used within 1 h. Cytological and chemical analysis showed a highly homogeneous nuclear preparation (DNA/RNA/protein, $1.0:0.12:3.2$) with insignificant cytoplasmic contamination.

Determination of DNA template activity. The template activity of DNA, either naked, heat-

denatured or in isolated nuclei, was assayed with DNA polymerase by means of the DNA-dependent incorporation of [^3H]dTMP into an acid-insoluble product. The standard assay system for *E. coli* DNA polymerase contained the following constituents in a final volume of 0.1 ml: $10 \mu\text{mol}$ of Tris-HCl, $\text{pH } 7.4$ at 37°C ; $0.7 \mu\text{mol}$ of MgCl_2 ; $0.1 \mu\text{mol}$ of β -mercaptoethanol; 20 nmol each of dCTP, dGTP and dATP; 20 nmol of dTTP containing $1 \mu\text{Ci}$ of [$\text{methyl-}^3\text{H}$]dTTP (Amersham-Searle, Arlington Heights, Ill., U.S.A.; 57 Ci/mmol); 0.2 unit of DNA polymerase; 0 – $10 \mu\text{g}$ of the appropriate DNA template; 0 – 5 mM polyelectrolyte. The mixture was incubated at 37°C for 60 min and assayed by the filter-paper-disc procedure of Bollum (1966). After appropriate washing, the radioactivity on the disc was determined by scintillation counting in 10 ml of toluene-based scintillation fluid. The same assay conditions were used for *M. lysodeikticus* DNA polymerase reactions.

When calf thymus DNA polymerase was used, the system contained the following constituents in a final volume of 0.1 ml: $4 \mu\text{mol}$ of potassium phosphate, $\text{pH } 7.0$ at 37°C ; $0.8 \mu\text{mol}$ of MgCl_2 ; $0.1 \mu\text{mol}$ of β -mercaptoethanol; 10.0 nmol each of dCTP, dGTP and dATP; 10 nmol of dTTP containing $0.5 \mu\text{Ci}$ of [$\text{methyl-}^3\text{H}$]dTTP; 0.2 unit of calf thymus DNA polymerase; 0 – $10 \mu\text{g}$ of the appropriate DNA template; 0 – 5 mM polyelectrolytes.

Electron microscopy. Nuclei were treated in buffer A with 4 mM-amine . After 30 min at 37°C the nuclei were centrifuged into a pellet. The pellets were fixed for 2.5 h in 2% glutaraldehyde in 'phosphate' buffer [$122 \text{ mM-NaH}_2\text{PO}_4$ ($\text{pH } 7.4$ at 25°C)- 58 mM-sucrose - 0.45 mM-CaCl_2]. After rinsing in the same buffer, they were post-fixed in 1% OsO_4 in the 'phosphate' buffer. All material was embedded in Araldite and ultrathin sections were prepared with the Porter-Blum ultramicrotome. The thin sections were stained with uranyl acetate and lead citrate and were examined with a Phillips 300 electron microscope.

Amines. The amines used were the salt forms: putrescine dihydrochloride; spermidine trihydrochloride; spermine tetrahydrochloride; methylglyoxal bis(guanyldiazotane) dihydrochloride monohydrate. Care was taken to store the amines at -20°C in a desiccator with desiccant. The amines were prepared as 10 mM solutions in water and adjusted to $\text{pH } 7.4$ as necessary. Fresh solutions were prepared for most of the analyses. In some experiments, twice-recrystallized polyamines were used and gave the same results as unrecrystallized material. In several instances the concentrations of the stock solutions of putrescine, spermidine and spermine were compared with those of recrystallized, dried standards by analysis on a Beckman 120C amino acid analyser (Marton *et al.*, 1973). According to this analysis,

the test solutions were within 10% of the concentration at which they were prepared.

Results

DNA polymerase activity

Fig. 1 shows the dependence of [³H]dTMP incorporation on the amount of heat-denatured DNA and enzyme. At 2 units/ml all of the enzymes examined were saturated by 50–100 μg of DNA/ml and thus all subsequent experiments were performed

under these conditions. At higher DNA concentrations the reactions were inhibited. This was especially apparent for the calf thymus enzyme, for which 200 μg of DNA/ml often resulted in a 30–50% decrease in incorporation.

These DNA polymerase reactions *in vitro* have been shown to depend on Mg²⁺ and enzyme concentration as well as on DNA, and to be linear for at least 90 min at 37°C. In addition it has been shown that the product of the reaction is sensitive to deoxyribonuclease, but not sensitive to ribonuclease or 0.3 M-KOH (Brown & Coffey, 1972). None of the amines could replace Mg²⁺ in the DNA-primed reaction with *E. coli* fraction IV polymerase.

Table 1. Inhibition of DNA polymerase activities by polyamines or methylglyoxal bis(guanylylhydrazine)

DNA polymerase assays were performed as described in the text. Control assays contained heat-denatured calf thymus DNA (8.5–10 μg) and enzyme (0.2 unit), but no drug, and incorporated 4000–8000 c.p.m.

Amine added	Concn. of amine (mM)	DNA polymerase activity (% of control)		
		<i>E. coli</i> fraction IV	<i>E. coli</i> fraction VII	Calf thymus
None (control)		100	100	100
Putrescine	0.05	104		
	0.1	107		100
	0.3	115		130
	0.5	112	97	
	1.0	100	94	87
	2.0	104	84	
	3.0	122	64	58
Spermidine	5.0	112		65
	0.05	118		
	0.1	136		103
	0.3	131		112
	0.5	149	112	97
	1.0	159	107	72
	2.0	159	73	
Spermine	3.0	166	47	21
	5.0	118		16
	0.05	153		
	0.1	153		106
	0.3	180		80
	0.5	172	93	
	1.0	115	46	51
Methylglyoxal bis(guanylylhydrazine)	2.0	38	12	
	3.0	31	3	8
	5.0	15		12
	0.05	129		
	0.1	108		63
	0.3	116		67
	0.5	98	60	
	1.0	77	35	28
	2.0	62	12	
	3.0	53	11	12
	5.0	43		14

Fig. 1. Activity of various DNA polymerases in the presence of heat-denatured calf thymus DNA

Assays were performed for 60 min at 37°C as described in the text, by using 0.2 unit of enzyme in each assay. The results are expressed as pmol of [³H]dTMP incorporated. The *E. coli* DNA polymerase (■) was fraction IV. ●, *M. lysodeikticus* DNA polymerase; △, calf thymus DNA polymerase.

Fig. 2. Effect of polyamines or methylglyoxal bis(guanylylhydrazine) on the DNA template activity of heat-denatured DNA in the presence of *E. coli* DNA polymerase fraction VII

Experimental conditions are described in the text. The results are expressed as a percentage of the control value. The 'control' assay (----) contained heat-denatured DNA (10 μg) and enzyme (0.2 unit) but no amine. None of the amines displayed template activity when assayed in the absence of DNA. ○, Putrescine; △, spermidine; ■, spermine; ●, methylglyoxal bis(guanylylhydrazine).

Table 2. *Effect of increasing DNA or enzyme on the DNA polymerase reaction in the presence of 3 mM-amine*

DNA polymerase assays were performed as described in the text. Assays contained 9 μ g of DNA and 0.2–0.6 unit of enzyme.

Additions	DNA (μ g/ml)	DNA polymerase activity (pmol of [3 H]dTMP incorporated/60 min)					
		<i>E. coli</i> fraction VII Enzyme added (unit/assay)			Calf thymus Enzyme added (unit/assay)		
		0.2	0.4	0.6	0.2	0.4	0.6
No amine	90	209	720	1200	107	105	55
	180	240			77		
Putrescine (3 mM)	90	122	942	1740	42	15	11
	180	280			47		
	360	350			90		
Spermidine (3 mM)	90	120	617	918	26	14	13
	180	222			30		
	360	199			53		
Spermine (3 mM)	90	13	58	114	6	10	8
	180	30			26		
	360	40			44		
Methylglyoxal bis(guanyldiazide) (3 mM)	90	42	137	293	16	12	11
	180	57			19		
	360	65			27		

Effect of amines on DNA polymerase activity

Fig. 2 shows the effects of different amines on *E. coli* DNA polymerase (fraction VII) in the presence of heat-denatured DNA. Spermine and methylglyoxal bis(guanyldiazide) both inhibited the reaction by more than 80% at 2 mM. Putrescine and spermidine were both much less effective inhibitors and at low concentration spermidine caused a slight stimulation.

Table 1 summarizes the data obtained by using three different DNA polymerase enzyme preparations at amine concentrations varying from 0 to 5 mM. The enzymes from calf thymus and *E. coli* (fraction VII) displayed comparable results in that they were inhibited slightly by putrescine or spermidine and strongly by spermine or methylglyoxal bis(guanyldiazide). On the other hand, the enzymes from *M. lysodeikticus* (not shown) and *E. coli* (fraction IV) were less susceptible to inhibition by the amines. With these two enzymes, putrescine or spermidine had little effect, or actually stimulated activity, and spermine or methylglyoxal bis(guanyldiazide) were not as effective inhibitors. For *E. coli* DNA polymerase (fraction IV) spermidine markedly activated the reaction, whereas spermine was the most effective inhibitor. The marked inhibition of the calf thymus enzyme by the amines was somewhat surprising considering the differences in specific activity of the four enzyme preparations; however, the calf thymus enzyme appears to be generally more sensitive to polyelectrolytes than are the enzymes from the other sources (Brown, 1973; D. G. Brown & V. L. Hansel, unpublished work).

In general, for each analysis, the buffer, salt, reducing agent and deoxyribonucleotide triphosphates were mixed together and then the DNA, polyamine and enzyme were added as the final three components. Essentially the same results were obtained regardless of the order of addition of these last three components. At and above 3 mM-spermine or -methylglyoxal bis(guanyldiazide), a visible precipitate formed in the assay tube when the amine was mixed with the DNA; in spite of the precipitate there was considerable activity in these samples.

The ability of naturally occurring polyamines to interact with nucleic acids has been known for some time. It has been reported that at saturation DNA binds approx. 1 molecule of spermine per 20 nucleotides (Hirschman *et al.*, 1967) or 1 molecule of methylglyoxal bis(guanyldiazide) per 6–8 nucleotides (Sartorelli *et al.*, 1965). Under the conditions used here, 3 mM-amine represents about a 10-fold molar excess over the amount of nucleotide present.

Since spermine or methylglyoxal bis(guanyldiazide) inhibited the reaction with *E. coli* DNA polymerase fraction VII, it was decided to determine if the inhibition could be overcome by adding either additional DNA or additional enzyme. Table 2 shows the results of these experiments. In the presence of the *E. coli* enzyme, the inhibition by putrescine or spermidine could be overcome by the addition of more DNA or more enzyme. In this experiment there was even some stimulation by putrescine at the higher enzyme level. On the other

hand, in the presence of spermine or methylglyoxal bis(guanyldrazone) there was only a slight to moderate increase in incorporation in the presence of additional enzyme and almost no increase in the presence of additional DNA.

With the calf thymus enzyme, additional polymerase resulted in no increase in incorporation, whereas additional DNA did restore some of the activity in the presence of spermine or spermidine and nearly all of the activity in the presence of putrescine. Since increased amounts of calf thymus enzyme appear to inhibit the normal reaction *in vitro* (see Table 2, 'No amine') it is not surprising that excess of enzyme in the presence of amines did not restore incorporation activity. However, if the polyamine binds to and eliminates some of the DNA from the reaction, the addition of more DNA could increase the [^3H]dTMP incorporation by the calf thymus polymerase even though the absolute amount of DNA in the assay exceeds the optimum for the reaction.

With *E. coli* fraction VII enzyme, additional DNA or enzyme was not inhibitory in the standard reaction (no amine). Thus it was surprising to observe that additional DNA had little effect in overcoming the inhibition by spermine or methylglyoxal bis(guanyldrazone). One interpretation of these results could be that these amines bind strongly to both the DNA and to the enzyme and may prefer the enzyme if it is available. Under these conditions, additional DNA or enzyme would have little effect in stimulating the reaction until sufficient enzyme had been added to bind all of the amine.

When similar experiments were performed with *E. coli* DNA polymerase fraction IV, a much less purified enzyme preparation, additional DNA or enzyme readily stimulated the reaction. Perhaps other protein components of the fraction IV preparation were capable of binding the amines thereby allowing the DNA-enzyme reaction to proceed.

Effect of amines on histone-nucleates

When isolated calf thymus histones were added to purified calf thymus DNA a precipitate resulted and the template activity of the DNA towards DNA polymerase was inhibited (Fig. 3). Complete inhibition was achieved at a ratio of histone/DNA of 1.5–2:1 (w/w). In an attempt to determine if the template activity of such a histone-nucleate could be stimulated by amines, a point at which about 60% of activity had been eliminated was selected for further study. Increasing concentrations of amines were then added to this DNA-histone complex and the template activity was measured by using DNA polymerase. Fig. 4 shows the results of the experiment using *E. coli* fraction VII polymerase. None of the amines could re-activate the template

Fig. 3. Inhibition of template activity of calf thymus DNA by calf thymus histone

Increasing amounts of unfractionated calf thymus histone were added to assay tubes containing DNA (100 μg/ml). The tubes were mixed and then left for 10 min at 0–4°C before the enzyme (fraction VII) was added and the incubation began. The arrow indicates the point at which the template activity was about 40% of the 'control' (----).

Fig. 4. Effect of polyamines and methylglyoxal bis(guanyldrazone) on the template activity of DNA partially inactivated by calf thymus histone

Calf thymus DNA (100 μg/ml) was mixed with unfractionated calf thymus histone (150 μg/ml) so that the reaction was 60% inhibited (see arrow in Fig. 3). Amines were added to this mixture and incubated as described in the text with 0.2 unit of *E. coli* DNA polymerase, fraction VII. ○, Putrescine; △, spermidine; ■, spermine; ●, methylglyoxal bis(guanyldrazone).

properties. In a similar experiment using *E. coli* fraction IV DNA polymerase, the initial template activity of this histone-nucleate was only 8% of that of DNA, but again none of the amines, tested from 0.05 to 5 mM, could overcome the inhibition.

Effect of amines on isolated rat liver nuclei

Isolated rat liver nuclei show very little or no template activity when assayed as described above. To determine if the amines might interact with the chromatin material of cell nuclei in such a way as to stimulate DNA synthesis, isolated nuclei were tested for their template activity *in vitro* by using *E. coli* DNA polymerase fraction VII and fraction IV. None of the amines, when tested at 0.05, 0.1, 0.5, 1, 3 or 5 mM, resulted in stimulation of [³H]dTMP incorporation (see Table 3). This was in contrast with our previous studies, in which negatively charged polyelectrolytes such as heparin or RNA resulted in dramatic increases in template activity (Brown & Coffey, 1971a,b, 1972, 1973).

Previous results (Arnold *et al.*, 1972) have shown that polyelectrolytes can cause alterations in the ultrastructure of isolated cell nuclei. To assess whether amines could alter nuclear structure, isolated washed rat liver nuclei were treated with 4 mM-amine and examined in the electron microscope (Plate 1). Untreated nuclei (a) show: a nucleolus; chromatin located centrally, next to the nucleolus (perinuclear chromatin) and next to the nuclear membrane; numerous interchromatin granules. When treated with putrescine (b) there was almost no change, with the possible exception of the formation of a perinucleolar halo. When treated with spermidine (c), spermine (d) or methylglyoxal bis(guanylhydrazine) (e), there were progressive changes in various aspects of nuclear structure. In general there appeared to be a dispersion of clumped chromatin and nucleoli

and an enlargement and clumping of interchromatin granules. The order of effectiveness in causing these changes was methylglyoxal bis(guanylhydrazine) > spermine > spermidine > putrescine. Methylglyoxal bis(guanylhydrazine)-treated nuclei showed the greatest changes compared with the untreated nuclei. The chromatin clumps appear amorphous and lack the granular appearance seen in the control and putrescine-treated nuclei. Also demarcation between nucleolus and perinucleolar chromatin is lost and the interchromatin granules appear much larger and arranged into several clumps. One additional feature seen consistently in methylglyoxal bis(guanylhydrazine)-treated nuclei was the appearance of clusters of granules in the nucleoli. Such clusters were not seen in preparations treated with the other amines.

Discussion

Ultrastructural studies on the effects of polyamines have been performed by Gfeller *et al.* (1972), who studied the effects of the polyamines on the nucleoli of isolated rat liver cell nuclei. These authors found that when nuclei were isolated with spermidine or spermine the nucleoli were larger and less aggregated. Putrescine caused relatively little change in nucleolar structure. Our observations extend these findings to the whole nucleus. Treatment with 4 mM-spermidine, -spermine or -methylglyoxal bis(guanylhydrazine) in the presence of 5 mM-Mg²⁺ caused a generalized swelling and disaggregation of not only nucleoli but chromatin and interchromatin granules. The reason for the swelling and disaggregation of nuclear components by polyamines is not known but may involve competition with basic proteins for binding to DNA. Such a competition may result in weakening or elimination of the forces which hold the nuclear DNA in its condensed form (Agrell & Heby, 1971).

If indeed the basic proteins restricting DNA are released, there might be a concomitant increase in the availability of template to exogenous DNA polymerase. We were not able to detect such an increase

Table 3. Effect of polyamines or methylglyoxal bis(guanylhydrazine) on the DNA template activity of calf thymus DNA or isolated rat liver cell nuclei

DNA polymerase assays were performed as described in the text by using amine concentrations from 0.05 to 5 mM. ³H radioactivity was counted at approx. 30% efficiency, so that 16 c.p.m. represents about 1 pmol of [³H]dTMP incorporated. The enzyme was from *E. coli* (fraction IV).

Addition	[³ H]dTMP incorporation (net c.p.m./60 min)
No DNA added	0
Heat-denatured DNA (91 µg/ml)	6950
+3 mM-putrescine	8030
+3 mM-spermidine	9580
+3 mM-spermine	750
+3 mM-methylglyoxal bis(guanylhydrazine)	2326
Liver cell nuclei (130 µg of DNA/ml)	200
+3 mM-putrescine	170
+3 mM-spermidine	105
+3 mM-spermine	85
+3 mM-methylglyoxal bis(guanylhydrazine)	130
+ heparin (100 µg/ml)	5100

EXPLANATION OF PLATE I

Electron micrographs of hepatic cell nuclei treated with polyamines or methylglyoxal bis(guanylhydrazine)

Isolated hepatic cell nuclei were examined either untreated (a) or treated with 4 mM-putrescine (b), 4 mM-spermidine (c), 4 mM-spermine (d) or 4 mM-methylglyoxal bis(guanylhydrazine) (e). Various nuclear structures are indicated as follows: N, nucleolus; CC, condensed chromatin; IG, interchromatin granules; H, nucleolar halo; NM, nuclear membrane; G, granules. The magnification of all photographs was the same.

in template in this study. Therefore it seems possible that added polyamines only weaken or loosen the restrictions on DNA conformation but do not themselves allow for increased template activity. Other studies (G. E. Noud & D. G. Brown, unpublished work) indicate that the nuclear DNA is markedly nicked during the nuclear isolation procedure yet does not serve efficiently as a template. If the amines could release the DNA template restrictions there would probably be sufficient starting points for polymerase action (Brown & Coffey, 1972).

As far as we are aware there are only five reports on the effects of polyamines on DNA polymerases, and the results are somewhat in conflict with those presented here. O'Brien *et al.* (1966) studied the effect of spermine on partially purified *E. coli* DNA polymerase in the presence of purified DNA. Spermine slightly stimulated the DNA-dependent reaction at concentrations below 0.05 mM but strongly inhibited the reaction at and above 0.1 mM. Brewer & Rusch (1966) used isolated nuclei from *Physarum polycephalum* as their DNA polymerase source and assayed its activity in the presence of exogenous DNA. Spermine, up to a concentration of 0.57 mM, was found to stimulate the reaction, whereas higher concentrations inhibited the reaction. In the presence of spermine but without exogenous DNA there was essentially no stimulation even though the enzyme was presumably present and active. From their data Brewer & Rusch (1966) concluded that the DNA-synthetic capacity of the isolated nuclei was not directly controlled by the DNA polymerase but probably resided with the structure or availability of the template itself.

Schwimmer (1968) used *E. coli* DNA polymerase to study the effects of putrescine, cadaverine and glyoxal bis(guanylhydrazone) on the template activity of free DNA and chromatin. At 1 mM, putrescine or cadaverine, but not glyoxal bis(guanylhydrazone), inhibited activity if DNA was used as template-primer, but with chromatin all compounds stimulated the reaction. These data are consistent with the idea that the amines interact with the chromatin DNA and displace basic proteins which may be associated with it.

Chiu & Sung (1972) studied the effect of polyamines on the DNA polymerases of rat brain. In a brain extract, putrescine, spermidine or spermine stimulated DNA polymerase activity by more than 50%, with the order of stimulation being spermine > spermidine > putrescine. By using the isolated polymerases, it was found that spermidine had no effect on polymerase A, but stimulated the activity of partially purified DNA polymerase B assayed with DNA as template. Above 1 mM, spermidine inhibited the reaction. In the presence of calf thymus nucleohistone, spermidine was less capable of stimulating activity, and when DNA-histone com-

plexes were used spermidine first stimulated and then inhibited the reaction. From their data, Chiu & Sung (1972) suggested that the stimulatory effect of spermidine was probably not due to the removal of histones but more likely to a stabilizing effect on the enzyme complex.

Our hypothesis was that polyamines might interact with DNA-protein complexes, compete with the protein for the DNA and thus release the restrictions from the template DNA. From the results presented, it is clear that polyamines do interact with isolated cell nuclei, and may cause marked alterations in nuclear ultrastructure. In addition, individual amines may stimulate and/or inhibit the activity of DNA polymerase preparations when assayed with purified naked DNA. However, the inability of the amines to stimulate the template activity of DNA in histone-DNA complexes or in isolated cell nuclei leads us to conclude that they probably have little direct effect in releasing template restrictions and thereby stimulating DNA synthesis *in vivo*. On the other hand it is quite possible that individual amines or a balance among amines may act to stimulate the polymerase enzyme activities in the cell.

Methylglyoxal bis(guanylhydrazone) has been studied extensively during the past year or two as a specific inhibitor of *S*-adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972; Pegg *et al.*, 1973; Pegg, 1973). Studies by Kay & Pegg (1973), Heby *et al.* (1973) and Corti *et al.* (1974) indicate that methylglyoxal bis(guanylhydrazone) may have more widespread actions in altering protein synthesis or other cellular functions. Our results show that methylglyoxal bis(guanylhydrazone) is an effective inhibitor of DNA polymerase *in vitro*. It is possible that part of the effectiveness of methylglyoxal bis(guanylhydrazone) as an anti-neoplastic agent is due to its ability to inhibit DNA synthesis and to disrupt chromatin structure.

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