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THE INFLUENCE OF SPERMIDINE ON THE REACTION OF RNA NUCLEOTIDYLTRANSFERASE

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SUMMARY

1. RNA synthesis, catalyzed by RNA nucleotidyltransferase (*Escherichia coli* B) and directed by native, double-stranded DNA, is significantly increased by spermidine.

2. By use of doubly labeled ATP ([8-^{14}C]- and [γ-^{32}P]-) and by zone centrifugation, we could show that, in the presence of spermidine, more RNA chains are initiated.

3. DNA irradiated with X-rays or ultraviolet light does not change the effect of spermidine on RNA synthesis. However, heat-denatured DNA prevents this influence.

4. In the case of poly A synthesis, no interference by spermidine can be observed.

5. The stimulation by spermidine is considerably lower when a more purified enzyme fraction was used.

INTRODUCTION

Spermidine and other polyamines are present in many tissues[1,2]; their concentration is dependent on the condition of the cell. Regenerating rat liver, for example, contains more spermidine than a normal liver[3], and growth hormone, which stimulates RNA and protein synthesis in the liver, leads to an increase of spermidine, too[4]. However, the function of polyamines in the metabolism is rather unknown. It has been observed *in vitro* that polyamines stimulate the RNA synthesis, which is catalyzed by RNA nucleotidyltransferase and primed by native, double-stranded DNA[5–7].

We have become interested in this stimulation of RNA synthesis; for our investigations, we used spermidine. In order to examine whether spermidine prolongs the single RNA chains or multiplies them, we worked with doubly labeled ATP. The RNA synthesis was measured by the incorporation of [8-^{14}C]AMP; we determined the number of RNA chains by the incorporation of [γ-^{32}P]ATP[8–11]. As templates we used differently treated DNA's. Besides RNA synthesis, we studied poly A synthesis in the same system.

MATERIALS AND METHODS

RNA nucleotidyltransferase (EC 2.7.7.6) was prepared from *Escherichia coli* B according to the procedure of CHAMBERLIN AND BERG[12]. Normally Fraction III (50-fold purified) was used; if Fraction IV (150-fold purified) was applied, it was specially indicated. DNA was isolated from fresh calf thymus by the method of KAY, SIMMONS AND DOUNCE[13]. DNA was irradiated with X-rays (^{60}Co γ-rays (500 μg/ml, 0.01 M NaCl, pH 7.0)) or with ultraviolet light as described in ref. 14. The doses are indicated in the tables. Heat-denatured DNA was obtained by heating the DNA (200 μg/ml, 0.01 M NaCl, pH 7.0) at 90° for 10 min and rapid cooling in ice.

The incubations at 37° were started by the addition of enzyme and terminated by the addition of 0.2 ml of a bovine serum albumin solution (0.3 %) and 5 ml ice-cold trichloroacetic acid (5 %). The samples were kept in ice for 10 min and centrifuged afterwards. The pellets were dissolved in 0.3 ml NaOH (0.2 M) and once more precipitated with 5 ml trichloroacetic acid. This washing procedure was repeated twice. The pellets were than collected on membrane filters (Sartorius MF 50), dried and, after addition of 10 ml scintillation solution (4 mg 2,5-diphenyloxazole and 0.1 mg 1,4-bis-(5-phenyloxazolyl-2)benzene per ml toluene), counted simultaneously in a Packard TriCarb liquid-scintillation counter.

The triphosphates ATP, GTP and UTP were purchased from Boehringer, Mannheim; [8-^{14}C]ATP, ADP and CTP from Schwarz BioResearch, Orangeburg, N. Y.; [γ-^{32}P]ATP from Amersham, England; spermidine·3 HCl from Calbiochem, Los Angeles.

RESULTS

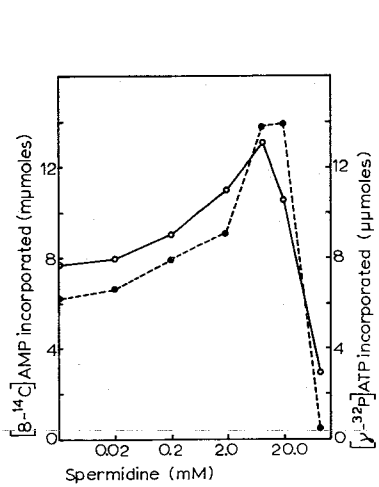
RNA synthesis

Influence of spermidine. In Fig. 1 it can be seen that RNA synthesis, measured by the incorporation of [8-^{14}C]AMP, is significantly increased by spermidine. The maximum is accomplished by a concentration of 10 mM of spermidine; higher amounts inhibit the RNA synthesis.

In order to be able to determine the number of RNA chains by the incorporation of [γ-^{32}P]ATP we had to add ADP to the reaction mixture. This is due to the fact that an enzyme utilizing the phosphates of the γ-position of ATP to form polyphosphates was present in our enzyme fraction. The activity of this enzyme can be completely suppressed by the addition of ADP[15]. Under this condition it turns out that the increase of the incorporation of [γ-^{32}P]ATP corresponds approximately to the one of [8-^{14}C]AMP (Fig. 1).

After further purification of the enzyme to Fraction IV, we changed its amount in the incubation mixture. Instead of 100 μg Fraction III, we now added 20 μg enzyme Fraction IV. Table I shows that the stimulation by spermidine is considerably lower when enzyme Fraction IV has been used.

Kinetics under the influence of spermidine. Fig. 2 demonstrates the difference between the incorporation of [8-^{14}C]AMP and [γ-^{32}P]ATP. In the case of [8-^{14}C]AMP, an increase is obtained over a period of 20 min, whereas the incorporation of [γ-^{32}P]ATP slows down after 5 min. A spermidine concentration of 25 mM established 5 min



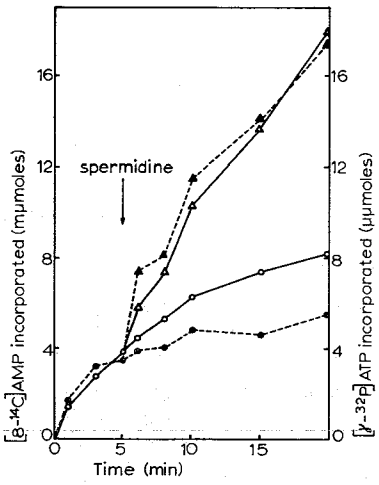


Fig. 1. Influence of spermidine on RNA synthesis. In a total volume of 0.25 ml were incubated for 20 min at 37°: 10 μmoles Tris buffer (pH 7.9); 1 μmole $MgCl_2$; 0.25 μmole $MnCl_2$; 2 μmoles 2-mercaptoethanol; 0.1 μmole GTP, UTP, CTP and ATP ([8-^{14}C]ATP: 190 counts/min per mμmole and [γ-^{32}P]ATP: 18 000 counts/min per mμmole); 0.08 μmole ADP; 100 μg enzyme (Fraction III); 10 μg native, double-stranded calf-thymus DNA; spermidine as indicated. ○–○, incorporation of [8-^{14}C]AMP; ●- - -●, incorporation of [γ-^{32}P] ATP.

Fig. 2. Kinetics of RNA synthesis under the influence of spermidine. The incubation mixture was the same as described in Fig. 1. Addition of spermidine (25 mM) as indicated. Without spermidine: ○–○, [8-^{14}C]AMP; ●- - -●, [γ-^{32}P]ATP. With spermidine: △–△, [8-^{14}C]AMP; ▲- - -▲, [γ-^{32}P]ATP.

TABLE I

INFLUENCE OF SPERMIDINE ON RNA SYNTHESIS USING ENZYME FRACTION IV

The incubation mixture was the same as described in Fig. 1, except for 20 μg of enzyme Fraction IV.

Spermidine (mM)	*Incorporation of*	
	[8-^{14}C]*AMP* (*mμmoles*)	[γ-^{32}P]*ATP* (*μμmoles*)
Control	5.15	10.50
0.04	6.10	10.60
0.20	6.19	11.70
0.80	6.82	16.10
2.00	3.69	13.00
4.00	2.50	8.00

after the start of the reaction, however, leads to a rapid increase of the incorporation of both [8-^{14}C]AMP and [γ-^{32}P]ATP. The same quantity of spermidine, present at the beginning of the incubation, surprisingly inhibits the RNA synthesis (Fig. 1).

Influence of spermidine using differently treated DNA. The results of our studies with differently treated DNA are summarized in Table II. The presence of spermidine during RNA synthesis directed by native, double-stranded DNA causes an incorporation of [8-^{14}C]AMP twice as high as the control and even three times higher values

TABLE II

INFLUENCE OF SPERMIDINE ON RNA SYNTHESIS USING DIFFERENTLY TREATED DNA

The incubation mixture was the same as described in Fig. 1. Spermidine (10 mM) as indicated.

Treatment of DNA		*Incorporation of*			
		[8-^{14}C]*AMP* (*mμmoles*)		[γ-^{32}P]*ATP* (*μμmoles*)	
		−Spermidine	*+Spermidine*	*−Spermidine*	*+Spermidine*
Native		6.23	12.06	8.10	23.50
X-rays	0.5 kR	5.07	11.07	8.30	25.90
	2.0 kR	3.82	7.59	9.10	25.00
	20.0 kR	0.60	1.55	6.85	12.60
Ultraviolet light	10 sec	2.99	7.23	5.46	16.00
	1 min	1.58	3.48	4.23	9.00
	30 min	0.42	0.96	2.46	2.70
Heat denaturation		6.80	5.90	27.90	37.20

in the case of [γ-^{32}P]ATP. Preceding treatment of the DNA with X-rays or ultraviolet light does not generally alter the increase caused by spermidine; the dose of irradiation is evidently without great importance. However, heat denaturation of the

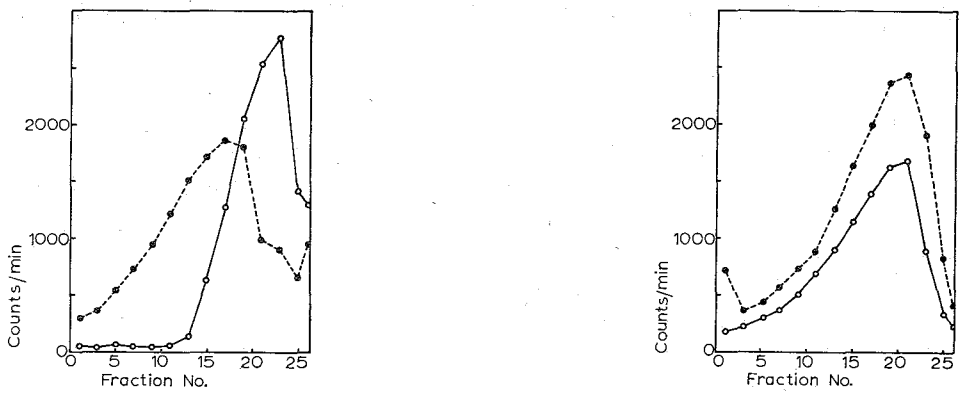


Fig. 3. Zone centrifugation of the enzyme–DNA–RNA complex. In a total volume of 0.5 ml were incubated for 20 min at 37°: 20 μmoles Tris buffer (pH 7.9); 2 μmoles $MgCl_2$; 0.5 μmole $MnCl_2$; 4.0 μmoles 2-mercaptoethanol; 0.2 μmole GTP, UTP, CTP, [8-^{14}C]ATP (3100 counts/min per mμmole); 200 μg enzyme; 20 μg DNA. In the case indicated, 8 mM spermidine was added. The incubation was terminated by cooling in ice. 0.2 ml of each sample was layered on 5 ml of a 5–20 % sucrose gradient containing 0.01 M Tris buffer (pH 7.9) and 0.1 M KCl. Centrifugation was performed at 4° for 5 h in a SW 39 Spinco rotor (20 000 rev./min). Fractions of 8 drops were collected, 0.03 ml serum albumin solution (0.3 %) was added, and the complex was precipitated by 5 ml cold trichloroacetic acid (5 %). The pellet was collected on a membrane filter and counted as described in MATERIALS AND METHODS. ○–○, control; ●- - -●, with spermidine.

Fig. 4. Zone centrifugation of the free RNA. The incubation mixture was the same as described in Fig. 3. Directly after incubation, 0.3 ml EDTA (0.033 M) and 0.2 ml sodium dodecylsulfate (4 %) were added to each sample. After another 5 min at 37°, 0.2 ml of the solution was layered on 5 ml of a 15–30 % sucrose gradient containing 0.01 M Tris buffer (pH 7.9) 0.1 M NaCl and 0.2 % sodium dodecylsulfate. Centrifugation was performed for 6 h at 25° in a SW 39 Spinco rotor (39 000 rev./min). ○–○, control; ●- - -●, with spermidine.

DNA suppresses the stimulating effect of spermidine on RNA synthesis almost completely. In our system, heat-denatured DNA also induces a 3–4-fold higher incorporation of [γ-^{32}P]ATP than the native, double-stranded one.

Zone centrifugation. It is known that the reaction catalyzed by RNA nucleotidyltransferase brings about a stable complex of enzyme–DNA–RNA[16]. We were interested in the influence of spermidine on the formation of this complex, and for this reason we performed zone centrifugation. Fig. 3 illustrates that the presence of spermidine causes a remarkable shift of the radioactivity to the heavier fractions. By the addition of EDTA and sodium dodecylsulfate after the reaction has taken place, RNA is, as is well known, released from the complex[9]. Performing this experiment we did not find any shift of the radioactivity to heavier fractions: in the presence of spermidine only, more activity is present in the single fractions (Fig. 4).

TABLE III

INFLUENCE OF SPERMIDINE ON POLY A SYNTHESIS USING DIFFERENTLY TREATED DNA

The incubation mixture was the same as described in Fig. 1, except for omission of GTP, UTP and CTP. Spermidine (10 mM) as indicated.

Treatment of DNA	*Incorporation of*			
	[8-^{14}C]*AMP* (*mμmoles*)		[γ-^{32}P]*ATP* (*μμmoles*)	
	−Spermidine	*+Spermidine*	*−Spermidine*	*+Spermidine*
Native	7.86	7.85	11.20	13.40
X-rays, 5 kR	12.30	12.10	23.50	25.60
Ultraviolet light, 10 sec	9.15	9.05	14.30	14.10
Heat denaturation	35.00	34.00	45.80	51.30

Poly A synthesis

The synthesis of poly A requires a template and, of course, ATP as the only triphosphate. If heat-denatured DNA is used as a template, the incorporation of [8-^{14}C]AMP and [γ-^{32}P]ATP is almost four to five times higher than in the case of native double-stranded DNA (Table III). DNA irradiated with X-rays (5 kR) significantly increases poly A synthesis. DNA treated with ultraviolet light (10 sec), on the other hand, only shows a slight stimulating effect. The presence of spermidine in these experiments does not substantially change the results.

DISCUSSION

The influence of spermidine on the reaction of RNA nucleotidyltransferase was studied with doubly labeled ATP. By the incorporation of [8-^{14}C]AMP we measured RNA synthesis in general, whereas the incorporation of [γ-^{32}P]ATP gave us the information on the number of RNA chains produced.

If native double-stranded DNA were used, spermidine stimulated both the incorporation of [8-^{14}C]AMP and [γ-^{32}P]ATP. With a more purified enzyme fraction, the stimulating effect of spermidine is diminished. If irradiated DNA (X-rays or ultraviolet light) is substituted for the native, double-stranded one, the stimulating

effect of spermidine on RNA synthesis is generally preserved. On heat denaturation of the DNA, on the other hand, the influence of spermidine is lost. From this one can conclude that the effect of spermidine is correlated with the double-stranded form of the DNA molecule.

In addition to RNA synthesis, we studied poly A synthesis in this system. The presence of spermidine does not change the formation of poly A; the DNA used as template is also unimportant in this case.

By use of zone centrifugation we could show that the enzyme–DNA–RNA complex, which is formed during the reaction of nucleotidyltransferase, is most probably enlarged under the influence of spermidine. However, releasing RNA from the complex by the addition of EDTA and sodium dodecylsulfate, the shift of the radioactivity to the heavier fractions did not occur. This may be due to the formation of more RNA chains by spermidine.

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