RNA POLYMERASE ACTIVITY IN PURIFIED NUCLEI FROM RAT PROSTATE GLAND IN THE PRESENCE OF POLYAMINES

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1. Introduction

It is well known that, among mammalian tissues, the prostate gland is one of the richest sources of spermine and spermidine [1, 2].

The levels of these two polyamines in the prostate gland are related to the functional activity of the gland itself which in turn is dependent on the circulating concentrations of androgenic steroids [3, 4]. On the other hand the nucleic acid metabolism in the prostate gland also depends on the blood levels of androgen hormones [5].

It has also been well established that a relationship exists between the levels of polyamines and the nucleic acid metabolism in a variety of experimental conditions ranging from the developing chick embryo [6] to the regenerating liver [7] and to bacterial cells [8].

Recently Moulton and Leonard [9] measured the changes in spermidine concentration in target organs in the presence and absence of sex hormones and concluded that the steroid-induced changes of spermidine levels may play an important role in the synthesis of certain types of RNA.

In the present report we present preliminary results concerning the action of spermine and spermidine on the DNA-dependent RNA polymerase activities in purified nuclei from intact rat ventral prostate.

2. Materials and methods

Male albino rats of the Wistar strain, 8 weeks old weighing 250 ± 10 g fed and maintained on laboratory standard conditions, have been used throughout all experiments.

The nuclei were purified from the gland by the method of Widnell and Tata [10] and were tested for the two DNA-dependent RNA polymerase reactions by using the conditions described by Tata and Widnell [11].

The reactions were stopped by the addition of 5 ml of ice-cold 0.5 N $HClO_4$ and 400 μg of RNA carrier.

The precipitates were washed twice with 5 ml of ethanol. The residues were dissolved in 0.5 ml of formic acid; 5 ml of ethylene glycol monomethyl ether were added followed by 10 ml of scintillator fluid [12].

DNA was determined spectrophotometrically after separation from RNA as indicated by Munro and Fleck [13].

3. Results and discussion

We have studied the two DNA-dependent RNA polymerase activities according to Widnell and Tata [10], since the products of the two activities are different in terms of base composition and nearest-neigh bour frequency.

Table 1 reports the in vitro effect of spermine or spermidine on DNA-dependent RNA polymerase activities in intact prostate nuclei. The Mg^{2+}-activated reaction shows an increased activity with all the concentrations of both spermine and spermidine assayed. A maximum level of activity is reached with a concentration of 0.9 mM of either spermine or spermidine, the highest level being reached with spermine. The

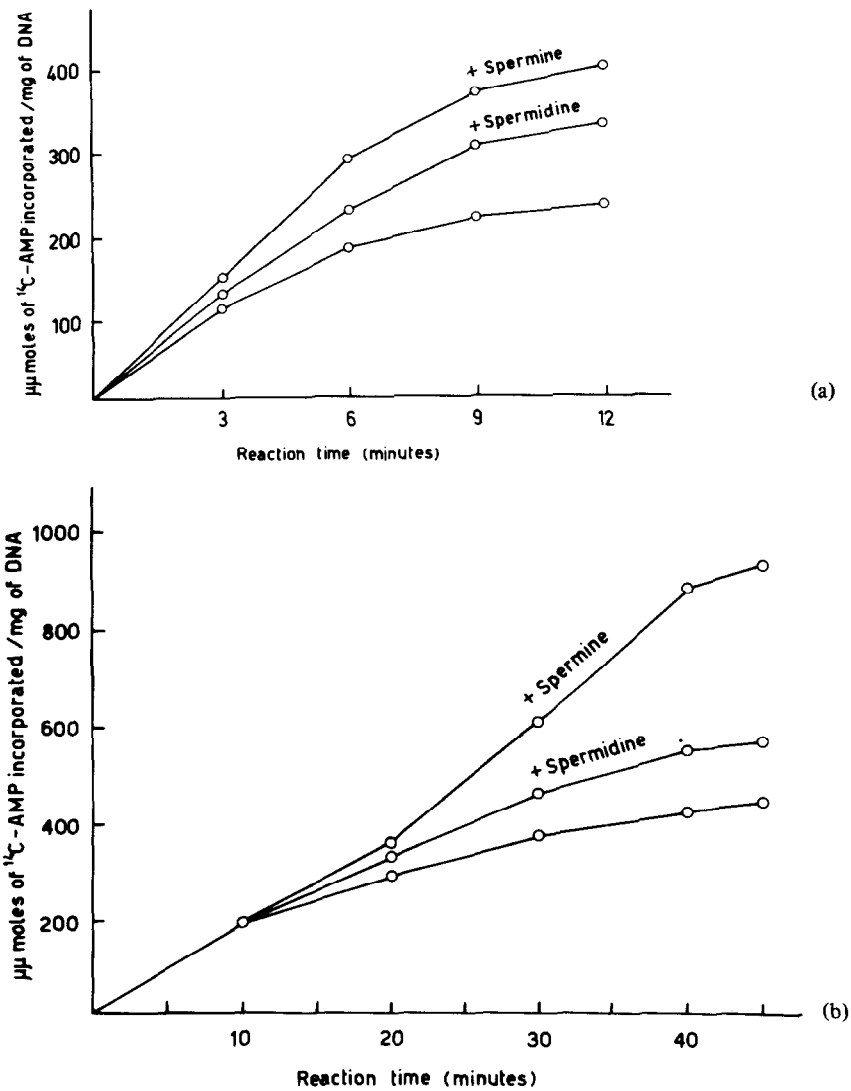

Fig. 1. Effect of spermine and spermidine on DNA-dependent RNA polymerase activities in rat ventral prostate nuclei. Spermine or spermidine (final concentration 0.9 mM) was added to the incubation medium, while no additions were made to the control. Mg^{2+}-activated reaction graph (a); $Mn^{2+}/(NH_4)_2SO_4$-activated reaction graph (b). The polyamine was added to the Mn^{2+}-activated reaction after 15 min of preincubation. The curves were plotted using average data from three determinations of pooled prostates (4 prostates per pool).

Table 1
Effect of spermine or spermidine, on DNA-dependent RNA polymerase activities in rat prostate nuclei.

Additions	Concentration of polyamine (mM)	RNA polymerase activity (percentage of control)	
		Mg^{2+}-activated reaction	$Mn^{2+}/(NH_4)_2SO_4$-activated reaction
None	control	100	100
Spermine	0.3	116	111
Spermine	0.6	138	146
Spermine	0.9	183	226
Spermine	1.2	157	149
Spermine	1.5	143	118
Spermine	2.0	119	112
Spermidine	0.3	114	108
Spermidine	0.6	141	118
Spermidine	0.9	153	134
Spermidine	1.2	137	122
Spermidine	1.5	135	108
Spermidine	2.0	117	105

Results are the averages of three determinations of pooled prostates (4 prostates per pool). Duplicate experiments agreed within ± 10%. Mg^{2+}-activated RNA polymerase activity in the control was 215 pmoles of ^{14}C-AMP incorporated/10 min/mg of DNA. Mn^{2+}-activated RNA polymerase activity in the control was 415 pmoles of ^{14}C-AMP incorporated/45 min/mg of DNA. (The specific activity of ^{14}C-ATP was 27 mCi/mmole).

$Mn^{2+}/(NH_4)_2SO_4$-activated reaction behaves in the same way, and is more sensitive to spermine.

Fig. 1 reports the pattern of the most effective concentration of polyamines (0.9 mM) during the reaction time. The action of the polyamines on the Mg^{2+}-activated reaction (fig. 1a) seems to start immediately after their addition to the incubation medium – the effect is already present after 3 min of incubation – and to continue throughout the reaction time. On the contrary, addition of spermine or spermidine to the mixture of the $Mn^{2+}/(NH_4)_2SO_4$-activated reaction (fig. 1b) results in an effect only after 10 min and from this point, higher incorporation is observed throughout the incubation time.

These results are of particular interest if we take into consideration the fact that the product of $Mn^{2+}/(NH_4)_2SO_4$-activated reaction according to Widnell and Tata [11] represents a more DNA-like RNA, and the highest effect of spermine is on this polymerase activity.

Our data show that the polycationic molecules studied in our experiments are able to stimulate the RNA polymerase activities assayed in whole nuclei purified from the rat prostate gland. It is interesting to point out that a very similar diphasic effect has been observed by Abraham [14] on the DNA-dependent RNA polymerase purified from *E. coli.*

At the moment it is very difficult to establish whether the polyamines act on the enzyme molecules, on the availability of DNA template or by stabilizing the reaction product itself. Further studies are in progress on this line.

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