SOLUBILIZED DNA-DEPENDENT RNA POLYMERASE FROM HUMAN PLACENTA: A Mn^{2+}-DEPENDENT ENZYME

H.-P.VOIGT, R.KAUFMANN and H.MATTHAEI

Arbeitsgruppe Biochemie, Max-Planck-Institut für experimentelle Medizin, 34 Göttingen, Germany

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The 100-fold purified RNA polymerase activity from human placenta is completely dependent upon added DNA. The enzyme is most active at 3 mM Mn^{2+} in the presence of 100 mM $(NH_4)_2SO_4$. Denatured DNA is a better template than native DNA. α-Amanitin completely inhibits the incorporation of ^{3}H-UMP, while rifampicin has no influence upon the enzymatic activity.

1. Introduction

Mammalian RNA polymerase has been solubilized from different tissues [1–13]. Stein and Hausen [13] have reported a fraction influencing template specifity of the purified enzyme from calf thymus. Others [14, 15] have demonstrated the solubilization of multiple forms of RNA polymerase activity from various tissues. The purpose of our work was to isolate and characterize a stable RNA polymerase activity from human placenta.

2. Materials and methods

Calf thymus DNA (Worthington), DEAE-cellulose DE 32 (Whatman), ATP, CTP, GTP (Boehringer), ^{3}H-UTP (Schwarz BioResearch), actinomycin D (Serva), rifampicin (Ciba). α-Amanitin was a generous gift of Prof. Th.Wieland, Heidelberg. Protein was determined according to Lowry et al. [16].

DNA from human placenta was prepared by the method of Marmur [17]. Denatured DNA was obtained by heating for 3 min in a boiling water bath and rapidly cooling in ice. RNA polymerase activity was assayed by the incorporation of ^{3}H-UMP into polynucleotides; 100 μl reaction mixtures contained: 100 mM tris acetate pH 7.9, 3 mM $MnCl_2$, 5 mM 2-mercaptoethanol, 400 mM $(NH_4)_2SO_4$ for fraction 1, and 100 mM $(NH_4)_2SO_4$ for fractions II–V, 1 mM each of ATP, CTP, GTP, 50 μM ^{3}H-UTP (265 Ci/mole), 12% glycerol, 10 μg calf thymus DNA, 5–10 μg enzyme fraction V proteins. Incubation time was 10 min at 37°. The reaction was stopped by heating for 1 min at 100°. 25 μl aliquots of the reaction mixtures were assayed as described [1, 2] for polynucleotide material not migrating in Whatman 3 MM paper upon elution with a 1:1 M ammonium acetate/95% ethanol mixture, adjusted to pH 5.5 with glacial acetic acid. For measurement of radioactivity see [2]. Counting efficiency for ^{3}H was 3.3%. Tissue and cell nuclei were prepared as described [2]. The following steps were varied. The nuclear pellet obtained after the second treatment with Triton X 100 was washed twice in 200 ml buffer A: 10 mM tris acetate pH 7.8, 10 mM $(NH_4)_2SO_4$, 6 mM $MgCl_2$, 1 mM K-EDTA, 5 mM 2-mercaptoethanol, 30% (v/v) glycerol; a final pH of 7.4 was adjusted with 2 M tris base. The pellet obtained after the second washing in this buffer was resuspended in 100 ml buffer A and stored at −30°.

3. Results and discussion

The previously published solubilization procedure [1, 2] was modified to obtain better yields of a purer enzyme with higher stability: the nuclear suspension (fraction I) was diluted with buffer A to 200 ml and

incubated for 15 min at 37°. After addition of $(NH_4)_2SO_4$ to a final concentration of 120 mM, incubation was continued for further 15 min. A few minutes after addition of the $(NH_4)_2SO_4$ a solid phase developed by coagulation of nucleoprotein material and the mixture was immediately and thoroughly homogenized to a homogenous suspension with an Esge homogenizer (Type M 100), 3–6 times for 45 sec with intermissions of 1 min, depending on viscosity. Insoluble nucleoprotein material was removed by 20 min centrifugation at 40,000 *g* at 0–4°. The supernatant (S-40) contained RNA polymerase fraction II, which was almost completely dependent on added DNA. Chromatography on DEAE-cellulose was performed as before [2]. Most of the polymerase activity was eluted between 300 and 400 mM $(NH_4)_2SO_4$ (fraction III). RNA polymerase was precipitated by addition of 350 g solid $(NH_4)_2SO_4$ per 1000 ml fraction III. The collected precipitate was dissolved in 1 to 2 ml buffer D: 10 mM tris acetate pH 7.8, 5 mM 2-mercaptoethanol, 50% (v/v) glycerol; final pH 7.4. It was dialysed for 12 hr against the same buffer (fraction IV). The supernatant of a 2 hr centrifugation of fraction IV at 100,000 g was called fraction V. This method reproducibly gives a roughly 100-fold purified enzyme fraction V with a specific activity of about 8000 units/mg protein* in a yield close to 15–20%

* 1 unit is defined as 1 pmole ^{3}H-UMP incorporation/min at 37° in the standard system.

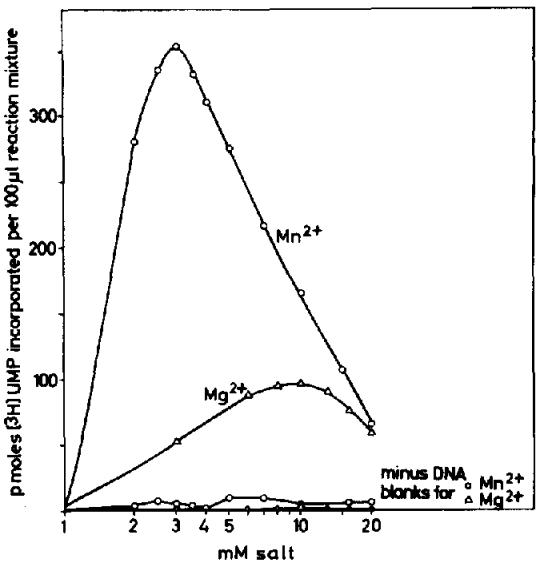

Fig. 1. Mn^{2+} and Mg^{2+} concentration curves: RNA polymerase was assayed at 100 mM $(NH_4)_2SO_4$ as described in Materials and methods.

(compared with the initial activity of nuclei see table 1).

Fractions IV and V can be frozen and stored for several weeks in liquid nitrogen with a loss of 10–20% of the initial activity. Further thawing and freezing does not lead to additional inactivation. Activity of the isolated RNA polymerase is strictly dependent

Table 1
Purification of DNA-dependent RNA polymerase from human placenta. For reaction conditions see Materials and methods.

Fraction	Preparation	Total protein (mg)	Total units		Specific activity
			+DNA	−DNA	
I Nuclei	A	1,020	50,000	50,000	49
	B	840	61,000	61,000	73
II S-40	A	360	19,060	2,130	53
	B	320	18,250	3,720	56.8
III DEAE-cellulose eluate	A	12.5	25,300	795	2,000
	B	11.7	19,307	270	1,650
IV Dissolved $(NH_4)_2SO_4$ precipitate	A	3.8	12,150	95	3,200
	B	3.4	14,825	410	3,800
V S-100	A	1.5	11,070	117	7,350
	B	1.7	14,375	264	8,450

Table 2
DNA dependency of the purified RNA polymerase activity fraction V. Reaction conditions see Materials and methods.

	^{3}H-UMP incorporated (pmoles/100 µl reaction mixture)
+Calf thymus DNA	421
–Calf thymus DNA	10
+Calf thymus DNA, denatured	979

upon added calf thymus DNA (see table 2). No significant differences are detected when saturating amounts of DNA from human placenta and calf thymus are compared. Heat denatured DNA reveals more than a two-fold higher template activity for RNA synthesis than does native DNA (see table 2). Omission of any one of the four ribonucleoside triphosphates causes a nearly complete inhibition of the enzymatic reaction.

Fig. 1 shows that the isolated RNA polymerase was much more active at the optimal concentration of Mn^{2+} (3 mM) than in the presence of the optimal Mg^{2+} concentration (10 mM), both tested in the presence of 100 mM $(NH_4)_2SO_4$. No significant RNA synthesis is found with Mg^{2+} (table 3) in the absence of $(NH_4)_2SO_4$. At present there is no evidence that application of our solubilization procedure to nuclei from human placenta yields any additional enzyme activity different from the one described here.

Table 3
Influence of $(NH_4)_2SO_4$, Mg^{2+} and Mn^{2+} on the RNA polymerase fraction V activity. Except for salt concentrations indicated, reaction conditions were as described in Materials and methods. The minus DNA blanks showed no variations compared to table 1.

No.	Salt (mM)			^{3}H-UMP incorporated (pmoles/100 µl reaction mixture)
	$(NH_4)_2SO_4$	$MgCl_2$	$MnCl_2$	
1	100	–	3	366
2	100	10	–	98
3	–	–	3	80
4	–	10	–	21
5	100	10	3	225
6	–	10	3	145
7	100	3	3	302

We have tested three antibiotics in respect to their inhibition of transcription activity of RNA polymerase from human placenta: actinomycin D, rifampicin, α-amanitin (table 4). Actinomycin D shows the well-known inhibition. Rifampicin is known to inhibit bacterial, but not mammalian RNA polymerase [18, 19]; this is confirmed in our system. α-Amanitin is a potent inhibitor at a concentration of 0.25 µg/ml, confirming the reports of Stirpe [22] and Seifart [20].

Application of the same procedure for purification of RNA polymerase from calf thymus resulted in an enzyme fraction IV with a specific activity of about 5000 units/mg protein. This enzyme showed similar

Table 4
Influence of antibiotics on transcription activity of RNA polymerase. For complete system see Materials and methods. The minus DNA blank was not influenced by these antibiotics.

	Inhibitor concentration (µg/100 µl)	^{3}H-UMP incorporated (pmoles/100 µl reaction mixture)
Complete system	–	570
–DNA	–	11
+ Actinomycin	1.0	90
	5.0	18
+ α-Amanitin	0.00125	250
	0.0050	53
	0.025	12
+ Rifampicin	1.0	554
	5.0	564

properties as the enzyme from human placenta: it was eluted at the same ionic strength from DEAE-cellulose, was strictly dependent on Mn^{2+} and $(NH_4)_2SO_4$, and preferred denatured DNA as template.

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