DNA-DEPENDENT RNA POLYMERASES FROM NORMAL MOUSE LIVER

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SUMMARY

Three forms of DNA-dependent RNA polymerase from adult mouse liver are separable by DEAE-Sephadex A-25 chromatography. Two of the forms (IA and IB) are insensitive to inhibition by α-amanitin, while the third form is completely inhibited by 0.3 μg/ml of α-amanitin. The three enzyme forms are compared to the enzymes found in adult rat liver, and to the enzymes found in several other mouse tissues.

INTRODUCTION

In 1964, Widnell and Tata (1) first reported the existence of multiple forms of RNA polymerase in eukaryotic organisms. These results were confirmed in 1969 when Roeder and Rutter (2) succeeded in isolating and characterizing two forms of RNA polymerase from rat liver nuclei. Since that time, experiments by Weaver et al. (3) on rat liver and by Kedinger et al. (4) on calf thymus, have yielded eukaryotic enzymes purified to homogeneity. The multiple forms of RNA polymerase in these two organisms are distinguished from each other by their elution profile upon DEAE-Sephadex chromatography and by their differential inhibition by the mushroom toxin, α-amanitin. According to the nomenclature of Kedinger et al. (5), calf thymus contains five forms of the enzyme, AI, AII, and AIII which are insensitive to inhibition by α-amanitin, and BI and BII which are sensitive to inhibition by α-amanitin. Forms AI and B, which are the major enzyme forms, (6,7) correspond to forms I and II in rat liver (8); form AIII corresponds to form III in rat liver (8).

We report here a study of the different forms of DNA-dependent RNA polymerases found in normal mouse liver nuclei. There is no report in the literature of the characterization of multiple forms of RNA polymerase from normal mouse

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liver, although several studies have been conducted on abnormal mouse cells (9,10) and on secondary mouse embryo cells (11). All of these studies have compared their enzymes to those of normal rat liver. We report here that there are significant differences among the polymerases found in mouse liver and rat liver. We also suggest that comparisons of normal and abnormal enzyme forms should be made within the same species.

MATERIALS AND METHODS

Mouse liver nuclei were obtained by the procedure described by Weaver et al. (3) for the isolation of rat liver nuclei. The nuclei were isolated from 30-40 g of liver obtained from adult CF1 mice (Carworth, Inc.). Further purification steps were based on procedures described by Roeder and Rutter (3,12,13). The enzymes was solubilized by adding 0.08 ml of 4 M ammonium sulfate (pH 7.9) to each ml of nuclear suspension, thus bringing the final concentration of ammonium sulfate to 0.3 M. The resulting, highly viscous, solution was sonicated for 1 minute total time, in 10 second bursts (Biosonik sonicator, large probe). Maximum voltage was used, and the solution maintained at 4° during sonication. Next, two volumes of TGMED buffer (0.05 M Tris-HCl, pH 7.9; 25% glycerol; 5 mM $MgCl_2$; 0.1 mM EDTA; 0.5 mM dithiothreitol) were added, and the solution centrifuged for 30 min. at 25,000 rpm (SW 36 rotor). This supernatant is, therefore, 0.10 M in $(NH_4)_2SO_4$. This enzyme will be referred to as fraction 4 enzyme. The enzyme was stored in liquid nitrogen.

The RNA polymerases from fraction 4 were separated by chromatography on DEAE-Sephadex, A-25, as described in the legend to Figure 1. Anywhere from 10-16 mg of fraction 4 protein were applied to a 0.9 x 12 cm column. The column was eluted with a linear $(NH_4)_2SO_4$ gradient from 0.1 to 0.8 M and 1 ml fractions were collected. Protein was determined by A_{280} or by the method of Lowry, et al. (14). DNA was determined by the diphenylamine reaction (15). $(NH_4)_2SO_4$ concentrations were determined by conductivity measurements.

RNA polymerase activity was determined by a modification of the procedure of Roeder and Rutter (2). The assay mixture contained 0.0125 μmole of unlabeled

UTP and 0.0002 μmole ^{3}H-UTP (22.2 Ci/mM, New England Nuclear) per 75 μl of solution. 50 μl of enzyme solution were used in each assay. When effects of α-amanitin (generously provided by Prof. T. Wieland, Heidelberg), rifampin (Calbiochem), or metal ions were studied, the appropriate concentration of effector was dissolved in 10 μl of TGMED buffer, and added to the enzyme for a 3 minute preincubation at 37°. Thus, the volume of a normal assay was 125 μl, and that of an assay to test an effector was 135 μl.

RESULTS AND DISCUSSION

Purification of the fraction 4 enzyme resulted in an approximately 4 fold increase in specific activity compared to the specific activity of the enzyme from the nuclear suspension. A typical specific activity (units/mg) of fraction 4 enzyme was 38.5. (One enzyme unit is defined as that amount of enzyme which catalyzes the incorporation of one picomole of ^{3}H-UMP into acid insoluble material in one minute.) The fraction 4 enzyme is stable for at least several months in liquid nitrogen. Activity of fraction 4 enzyme is >90% dependent on added exogenous DNA. Fraction 4 enzyme is completely insensitive to inhibition by rifampin, thereby eliminating the possibility of a mitochondrial contaminant (16).

DEAE-Sephadex A-25 chromatography of the fraction 4 enzyme resolved the enzyme into three peaks designated IA, IB and II (see Figure 1). The three peaks (IA, IB and II) were eluted from the column at ammonium sulfate concentrations of 0.10, 0.14, and 0.28 M respectively. The presence of the three peaks is constant among several different enzyme preparations. The exact $(NH_4)_2SO_4$ concentration at which the forms elute is also remarkably constant. Among nine different columns studied, the following range of $(NH_4)_2SO_4$ concentrations at which the forms eluted was: IA (0.10 M); IB (0.12-0.16 M); II (0.26-0.30 M). The heights of the peaks vary somewhat, but peak IB is always the least active form. Form II has a specific activity approximately 200 times greater than the whole fraction 4 enzyme. None of the forms has any activity in the

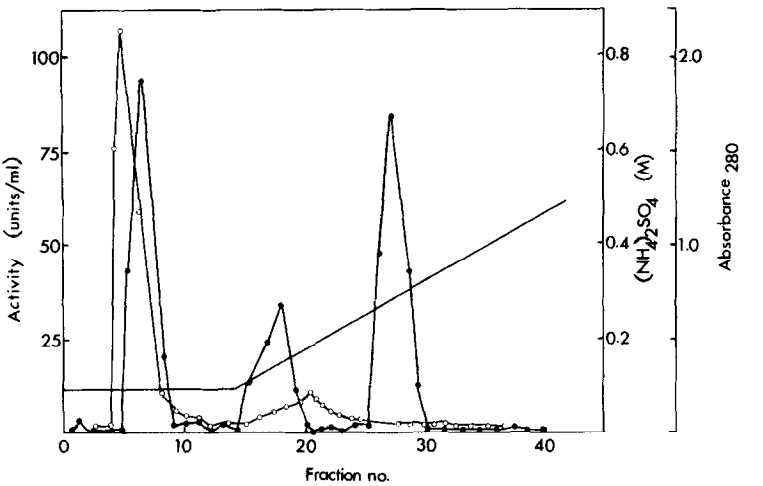

Figure 1. DEAE-Sephadex chromatography of RNA polymerase (fraction 4 enzyme). Fraction 4 enzyme (16.2 mg protein in 3 ml) was layered on a 0.9 x 12 cm DEAE-Sephadex A-25 column, washed with 8 ml of 0.10 M $(NH_4)_2SO_4$ in TGMED buffer, and eluted with a linear gradient of $(NH_4)_2SO_4$ in TGMED buffer. One ml fractions were collected and assayed as described in the text. Activity (•—•); Protein (A_{280}) (○—○); $(NH_4)_2SO_4$ concentration (—).

absence of divalent cations (Mn^{2+} or Mg^{2+}) or in the absence of exogenous DNA. Only form II is stable, for several weeks, to freezing and rethawing.

The properties of the three enzyme forms are summarized in Table I. Form IA elutes in the void volume, but is not due to overloading the column as shown by the fact that different amounts of protein added to the column (3 to 20 mg) do not change the elution pattern, and by the fact that peak IA shows no inhibition by α-amanitin while whole fraction 4 enzyme shows 42% inhibition at 0.03 μg/ml of α-amanitin and 50% inhibition at 0.30 μg/ml of α-amanitin. Furthermore, rechromatography of form IA results in a single peak of enzyme activity again eluting at 0.10 M $(NH_4)_2SO_4$.

Our forms IB and II seem to correspond to Roeder and Rutter's forms I and II (2) with respect to α-amanitin sensitivity and Mn^{2+}/Mg^{2+} activity ratios. The existence of our form IA does have some precedence when the elution profile

Table I. Metal Ion Optima and α-Amanitin Sensitivity of DNA-dependent RNA Polymerases from Normal Mouse Liver Nuclei

Experiment	Enzyme Form		
	IA	IB	II
Elution from DEAE-Sephadex by $(NH_4)_2SO_4$ (M)	0.10	0.14	0.28
Optimal Mn^{2+} concentration (mM)	1-2	1-2	1-2
Optimal Mg^{++} concentration (mM)	2.5-9	2.5-7	2.5-9
Ratio of activities at optimal ion concentrations (Mn^{2+}/Mg^{2+})	3.3	2.2	5.7
%Inhibition by α-amanitin 0.01 μg/ml	N.D.[a]	N.D.	72
0.03 μg/ml	7	0	97
0.05 μg/ml	N.D.	N.D.	97
0.10 μg/ml	0	N.D.	100
0.20 μg/ml	N.D.	N.D.	100
0.30 μg/ml	0	0	100

[a]N.D. = Not done

of our enzyme is compared to that of Jacob et al. for rat liver enzyme (17). These workers find a peak of enzyme activity in the void volume, which had not been previously detected in rat liver preparations. It is insensitive to α-amanitin, as is our form IA enzyme activity.

Thus, different laboratories working on RNA polymerases from the same tissue (rat liver) have described the detection of different numbers of RNA polymerase enzyme forms. The one point of agreement is that the most stable form is the α-amanitin-sensitive nucleoplasmic form [form II of Roeder and Rutter (2); form B of Jacob et al. (17)]. We too, find this to be true for mouse liver. We believe that descriptions of variable numbers of enzyme forms may be due to varying degrees of aggregation of the enzymes. For the mouse liver enzyme it is absolutely imperative to elute the column starting at 0.1 M $(NH_4)_2SO_4$ to get reproducible results. If the fraction 4 enzyme is dialyzed against 0.05 M $(NH_4)_2SO_4$ it becomes visibly opalescent and shows varying numbers of apparent enzyme forms after elution from a DEAE-Sephadex column.

We finally want to make the following suggestions with respect to four papers which have studied mouse RNA polymerase activities. First, Stirpe and Fiume (18) describe the effect of α-amanitin both in vivo and in vitro on isolated mouse liver nuclei. Although these workers never isolated their enzyme forms, they assumed that there were at least two forms based on differential Mn^{2+} or Mg^{2+} activiation of whole nuclei. Our results on isolated forms IA, IB and II correspond very well with these authors' observations that only the Mn^{2+} activated enzyme activity (corresponding to our form II) was inhibited by α-amanitin. Secondly, Lentfer and Lezius (9) describe an RNA Polymerase B from mouse-myeloma cells, the properties of which seem very similar to our form II enzyme. The third paper dealing with mouse RNA polymerase is the recent study of Blair and Dommasch (10) in which the polymerase from Ehrlich ascites tumor cells was examined. These authors conclude that they may have isolated an α-amanitin sensitive form of the enzyme unique to cancer cells. However, several objections to their study must be raised as follows: First, and most important, is that no control experiment is reported in which the elution pattern of normal mouse tissue, on DEAE-Sephadex is described. Second, a stepwise, not a linear $(NH_4)_2SO_4$ gradient is used; it is well known that stepwise gradients produce elution artifacts (19). Third of all, they base their new enzyme form on its sensitivity to α-amanitin at a concentration of 3 μg/ml. This concentration is ten times higher than that normally used to distinguish α-amanitin sensitive and insensitive forms of RNA polymerase. Perhaps the use of the well defined system described in this paper, applied to the ascites tumor cells, could help clarify some of these points.

Finally, multiple forms of RNA polymerase from secondary mouse embryo cells have been described by Monjardino and Crawford (11). Their isolation and chromatographic procedures are sufficiently different from ours that no valid comparisons can be made between their embryonic RNA polymerases and our adult RNA polymerases. However, with the data reported here, we are now in a position to evaluate any changes in the enzymes during early mouse development and to compare them to the normal adult mouse liver enzymes.

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