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Abstract

DNA-dependent RNA polymerases (EC 2.7.7.6) were extracted and partially purified form the nuclei of rat ascites hepatoma cells (AH-130) induced by 4-dimethylaminoazobenzene. The patterns of RNA synthesis and the properties of these enzymes were compared with enzymes from the nuclei of rat liver. The specific activity of RNA polymerase in the homogenate from the nuclei of AH-130 cells was the same as normal rat liver nuclei. RNA polymerase was solubilized from the homogenate at high ionic strength and separated into two forms by DEAE-Sephadex column chromatography. Enzymatic characterization showed that these enzymes corresponded to RNA polymerase I and II. RNA polymerase I more effectively transcribed native DNA than denatured DNA at low salt concentration, but at high salt concentration RNA polymerase I effectively transcribed denatured DNA. RNA polymerase II more effectively transcribed denatured DNA. In AH-130 cells the activity of RNA polymerase I was 4 to 5 times higher than RNA polymerase II, and in rat liver the activity of RNA polymerase I was 1.5 to 2 times higher than RNA polymerase II. The activity of RNA polymerase I in AH-130 cells may have increased by induction.

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A COMPARATIVE STUDY OF DNA-DEPENDENT RNA POLYMERASES FROM RAT ASCITES HEPATOMA CELL NUCLEI AND FROM RAT LIVER NUCLEI

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Abstract: DNA-dependent RNA polymerases (EC 2. 7. 7. 6) were extracted and partially purified from the nuclei of rat ascites hepatoma cells (AH-130) induced by 4-dimethylaminoazobenzene. The patterns of RNA synthesis and the properties of these enzymes were compared with enzymes from the nuclei of rat liver. The specific activity of RNA polymerase in the homogenate from the nuclei of AH-130 cells was the same as normal rat liver nuclei. RNA polymerase was solubilized from the homogenate at high ionic strength and separated into two forms by DEAE-Sephadex column chromatography. Enzymatic characterization showed that these enzymes corresponded to RNA polymerase I and II. RNA polymerase I more effectively transcribed native DNA than denatured DNA at low salt concentration, but at high salt concentration RNA polymerase I effectively transcribed denatured DNA. RNA polyemerase II more effectively transcribed denatured DNA. In AH-130 cells the activity of RNA polymerase I was 4 to 5 times higher than RNA polymerase II, and in rat liver the activity of RNA polymerase I was 1.5 to 2 times higher than RNA polymerase II. The activity of RNA polymerase I in AH-130 cells may have increased by induction.

The specificity of gene transcription is determined both by RNA polymerase and by the specific template restriction by protein and other factors. RNA polymerase I, II, and III have been separated from many eukaryotic organisms by DEAE-Sephadex chromatography (1, 2). RNA polymerase I is located in the nucleolus and is responsible for the synthesis of ribosomal RNA (3). RNA polymerase II and III are located in the nucleoplasm (4). Each of these multiple forms of RNA polymerase has distinct functions in genetic control, and the activities of these enzymes are influenced by hormones and other agents, such as phytohemagglutinin (5, 6, 7). Until the recent development of Roeder and Rutter (2) for effective extraction of RNA polymerase, the enzyme preparations from cancer cells had low recovery rates during extraction. In Reuber H-35 hepatoma cell line, RNA polymerase I and II activity was reported to be higher than in the normal rat liver (8). It seems that in Novikoff hepatoma and mouse myeloma cells (9, 10), RNA

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polymerase I activity was higher than RNA polymerase II activity. In KB cells (a neoplastic human cell line), adenovirus infected-KB cells (11), and Ehrlich ascites tumor cells (12), RNA polymerase activities have patterns similar to other normal mammalian cells. In most of these studies, appropriate comparable tissues were not used. In the present investigation, RNA polymerase I and II were solubilized from rat ascites hepatoma cells (AH-130) (13) induced by 4-dimethylaminoazobenzene, and these enzymes were compared to enzymes from normal rat liver of the same strain.

MATERIALS AND METHODS

Materials. Intraperitoneally injected AH-130 cells were grown in ascites fluid of albino rats of the Donryu strain. The cells were harvested 7 days after inoculation. Tritium labeled uridine triphosphate (H^3-UTP) was purchased from the Radiochemical Center, England; dithiothreitol and α-amanitin were from Calbiochem; unlabeled nucleotides were from Boelinger; and calf thymus DNA (type I) was from Sigma Chemical Co.

Isolation of nuclei. AH-130 cells were washed with phosphate buffered saline to eliminate red blood cells and suspended in 1.0% Triton X-100 solution containing 0.25 M sucrose; 5 mM $MgCl_2$; 10 mM Tris-HCl, pH 7.9; and 5 mM 2-mercaptoethanol and centrifuged at 170×g for 5 min. The pellet was suspended in 15 volumes of Triton solution and filtrated through four layers of cheese cloth. The filtrate was homogenized with Emanuel-Chaikoff homogenizer (14) with a narrow piston clearance (Takashima Shoten Co., Japan). The homogenate was washed three times by centrifugation at 170×g for 5 min in 0.25 M sucrose containing 10 mM Tris-HCl, pH 7.9; 5 mM $MgCl_2$; and 5 mM 2-mercaptoethanol. The crude nuclear pellet was suspended in 2.5 M sucrose containing 10 mM Tris-HCl, pH 7.9; 5 mM $MgCl_2$; and 5 mM 2-mercaptoethanol. The sucrose concentration was adjusted to 2.2 M by dilution with 0.25 M sucrose solution. Then, it was centrifuged at 45,000×g for 1 hr, and the purified nuclear pellet was collected.

Rat liver nuclei were isolated from albino rats of the Donryu strain. The rat liver was homogenized in a Teflon-glass homogenizer in 10 volumes of 0.4% Triton X-100 containing 10 mM Tris-HCl, pH 7.9; 10 mM $MgCl_2$; 5 mM 2-mercaptoethanol; and 0.25 M sucrose. The homogenate was washed and nuclei were purified as described earlier.

Preparation of the enzymes. RNA polymerases were solubilized from the purified nuclei and chromatographed by the procedure of Roeder and Rutter (2, 15). The purified nuclei were suspended in 1 M sucrose containing 50 mM Tris-HCl, pH 7.9; 5 mM $MgCl_2$; 0.1 mM EDTA; and 0.5 mM dithiothreitol (1 ml/g of tissue). The solution 4 M $(NH_4)_2SO_4$ (pH 7.9) was added with vigorous agitation to this suspension to bring the concentration to 0.3 M. After the suspension was stirred for 30 min at 0°C, it was diluted to 0.1 M $(NH_4)_2SO_4$ with buffer A (25% v/v glycerol; 50 mM Tris-HCl, pH 7.9; 5 mM $MgCl_2$; 0.1 mM EDTA; 0.1 mM dithiothreitol) and centrifuged at 105,000×g for 30 min. The

supernatant fraction was diluted to 0.033 M $(NH_4)_2SO_4$ and centrifuged again at 105,000×g for 30 min and the supernatant was immediately applied to DEAE-Sephadex A-25 column (1.6×8 cm) that was previously equilibrated with buffer A containing 0.03 M $(NH_4)_2SO_4$. After washing with this buffer, the column was eluted by 80 ml of linear gradient of 0.03-0.4 M $(NH_4)_2SO_4$ in buffer A. Finally the column was washed with 0.4 M $(NH_4)_2SO_4$ in buffer A. Each 1.8 ml fraction was collected, and 0.1 ml aliquots of the fraction were assayed for RNA polymerase activity.

RNA polymerase assay. The reaction mixture in a final volume of 0.2 ml contained 56 mM Tris-HCl, pH 7.9; 1.6 mM $MnCl_2$; 8 mM KCl; 1.6 mM 2-mercaptoethanol; 0.4 mM each of ATP, GTP, and CTP; 0.02 mM unlabeled UTP; 5-^{3}H-UTP 1 μCi (10-20 Ci/m mole); and 5 μg native calf thymus DNA. $(NH_4)_2SO_4$ and enzyme were added as indicated in the legends to the tables and figures. After incubation for 10 min at 37°C, the reaction was stopped at 0°C by adding 2 ml of 5% trichloroacetic acid containing 0.04 M sodium pyrophosphate. After 1 hour, the acid precipitable fraction was collected on a Whatman GF/C filter disc by suction. The filter was washed 4 times with 5 ml of the trichloroacetic acid solution, dried under an infrared lamp, and counted in a vial with 10 ml of toluene containing PPO and POPOP. One unit of enzyme was defined as the enzyme which incorporated 1 n mole of UTP into acid precipitable product in 10 min under standard conditions.

Protein was determined by Lowry's method after precipitation with 5% trichloroacetic acid (16).

RESULTS

RNA polymerase was solubilized almost completely from AH-130 cell nuclei and the solubilized enzyme activity was completely adsorbed on DEAE-Sephadex. The RNA polymerase was eluted at 0.13 M and 0.22 M of $(NH_4)_2SO_4$. The former was insensitve to 1 μg/ml of α-amanitin and the latter was completely inhibited by 1 μg/ml of α-amanitin (Fig. 1). These corresponded to RNA polymerase I and II, respectively. The specific activity for these enzymes were 2.2 and 1.5 units/mg protein, respectively, and these values were 65 and 44 times higher, respectively, than those of the initial nuclear extract (Table 1). The total activity of polymerase I per total activity of polymerase II was 4.

The time course of the reaction was linear for 30 min for both enzymes. The amount of synthesized RNA was proportional to the amount of added enzymes, and the activities of these enzymes were completely dependent on added DNA. The enzyme activities were sensitive to DNase I and pancreatic RNase. The activities were inhibited completely by actinomycin D but not inhibited by rifampicin SV which inhibits bacterial and mitochondrial RNA polymerases.

Effect of ionic strength. The reaction by the enzymes was markedly

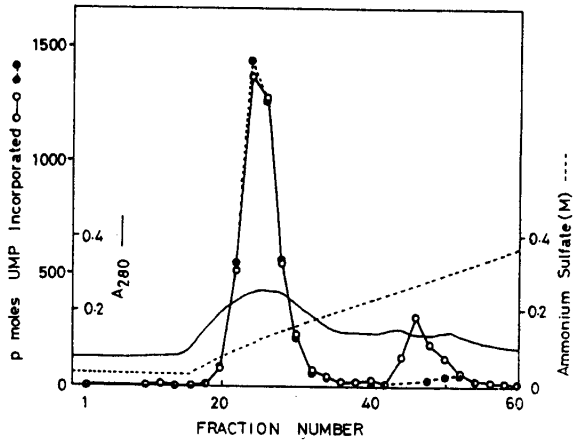

Fig. 1. DEAE-Sephadex chromatography of RNA polymerase from AH-130 cell nuclei. Solubilized protein (20 mg) from AH-130 cell nuclei was applied to a 1.6×8 cm column and eluted as described in Materials and Methods. Aliquots (0.1 ml) of each fraction were assayed for RNA polymerase under standard condition. ○——○, activity in standard condition; ●----●, activity in the presence of 1 μg/ml of α-amanitin.

TABLE 1. SUMMARY OF PURIFICATION OF RNA POLYMERASES FROM AH-130 CELL NUCLEI AND RAT LIVER NUCLEI

	Rat Liver			AH-130		
	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Total protein (mg)	Total activity (units)	Specific activity (units/mg)
Homogenate[a)]	74	8.0	0.108	51	5.4	0.1
Residue[a)]	31	1.5	0.049	27	0.51	0.02
Supernatant[a)]	36	10.8	0.315	20	5.4	0.27
Peak I[b)]		7.6	1.7		8.1	3.7
Peak II[b)]		3.4	2.9		1.5	2.5

a) The nuclear homogenate, supernatant, and residue of the extract were assayed at 0.05 M $(NH_4)_2SO_4$.

b) Peaks I and II were assayed at 0.04 M and 0.13 M $(NH_4)_2SO_4$, respectively.

Nuclei were isolated from rat liver and AH-130 ascites hepatoma cells. They were lysed in 0.3 M $(NH_4)_2SO_4$. The lysate was centrifuged at 105,000×g for 30 min and the supernatant was diluted to 0.03 M $(NH_4)_2SO_4$ with buffer A and applied to DEAE-Sephadex column. The fraction at each purification step was assayed for RNA polymerase activity by the indicated conditions. Peak I and peak II correspond to the activity peak of RNA polymerase I and II, respectively. One unit of enzyme corresponds to the enzyme activity which incorporates 1 n mole of UTP into acid precipitable product in 10 min.

influenced by ionic strength, as indicated in Fig. 2. Polymerase I had optimal activity at a concentration less than 0.05 M of $(NH_4)_2SO_4$, and polymerase II had optimal activity at 0.12 M. At concentrations higher than 0.2 M, both enzymes were strongly inhibited. These results coincide with those for rat liver RNA polymerases (2).

Effects of divalent cations. The optimal concentration of Mn^{++} for RNA polymerases from AH-130 cells was 1.5-2 mM (Fig. 3). The optimal concent-

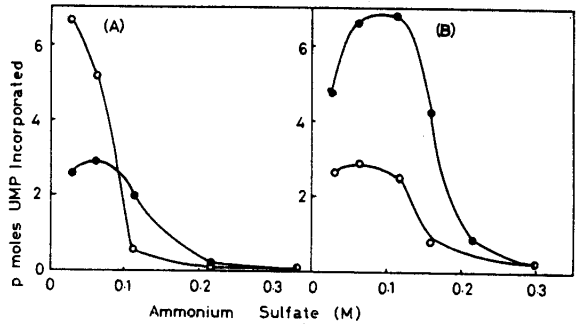

Fig. 2. Effects of ionic strength on the template preference of AH-130 nuclear RNA polymerases. (A), Activity of RNA polymerase I. (B), Activity of RNA polymerase II. RNA polymerase I and II from AH-130 nuclei were dialyzed against buffer A containing 0.03 M $(NH_4)_2SO_4$. Calf thymus DNA was dissolved in 10 mM Tris-HCl (pH 7.9), 10 mM NaCl, 0.1 mM EDTA, and denatured in a boiling water bath for 5 min. ○——○, RNA polymerase activity assayed with native DNA; ●——●, RNA polymerase activity assayed with denatured DNA.

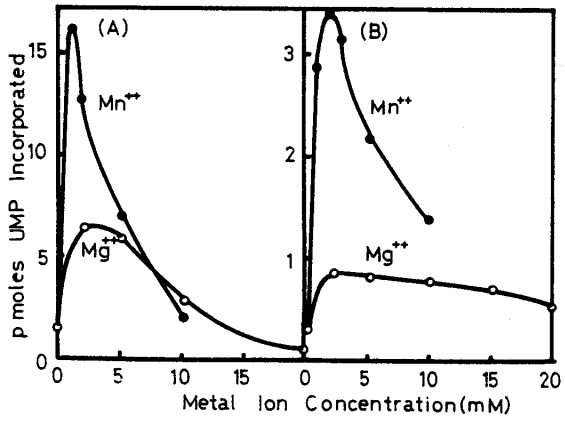

Fig. 3. Effects of Mg^{++} and Mn^{++} ion concentration on the activity of RNA polymerases from AH-130 nuclei. (A), Activity of RNA polymerase I. (B), Activity of RNA polymerase II. RNA polymerases were dialyzed against 50 mM Tris-HCl (pH 7.9), 25% v/v glycerol, 0.1 mM EDTA, and 0.1 mM dithiothreitol and assayed under the indicated conditions. ○——○, activity in the presence of Mg^{++}; ●——●, activity in the presence of Mn^{++}.

ration of Mg^{++} for RNA polymerase I was 5 mM and that for polymerase II was 2–15 mM. The ratio of the activity assayed at optimal Mn^{++} concentration to the activity assayed at optimal Mg^{++} concentration was 2.4 for RNA polymerase I and 3.9 for polymerase II. The Mn^{++}/Mg^{++} activity ratio for polymerase II from AH-130 cells was lower than for RNA polymerase II from normal rat liver nuclei.

Template requirement. At optimal ionic strength, RNA polymerase I activity with native DNA as template was higher than the activity with denatured DNA. The activity of RNA polymerase II with denatured DNA was higher than the activity with native DNA. The template preference of RNA polymerase I from AH-130 cells was influenced by ionic strength (Fig. 2). At concentrations under 0.09 M $(NH_4)_2SO_4$, RNA polymerase I from AH-130 cells transcribed native DNA actively and at concentrations over 0.09 M it transcribed denatured DNA actively. On the other hand, RNA polymerase II transcribed denatured DNA more effectively than native DNA.

Comparison of RNA polymerases from AH-130 cell nuclei with RNA polymerases from rat liver nuclei. RNA polymerases from rat liver were extracted by stirring the nuclei in 0.3 M $(NH_4)_2SO_4$ and subjected to DEAE-Sephadex chromatography (Fig. 4). RNA polymerase I and II were separated and the specific activities were 1.7 and 2.9 units/mg of protein, respectively. The specific activity of polymerase I from AH-130 cell nuclei was about two times higher than from normal rat liver nuclei but the specific activity of RNA polymerase II from AH-130 cell was slightly lower than from rat liver. The

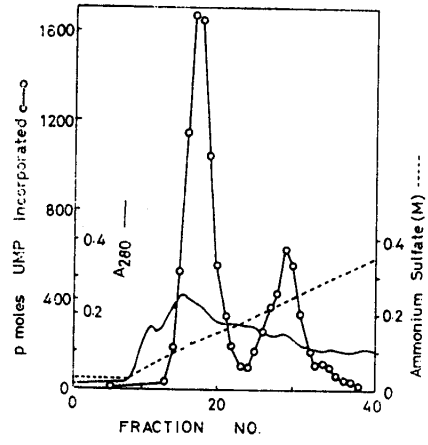

Fig. 4. DEAE-Sephadex chromatography of RNA polymerases from normal rat liver nuclei. Solubilized protein (36 mg) from rat liver nuclei was applied to a 1.6×8 cm column and eluted as described in Materials and Method. Aliquots (0.1 ml) of each fraction were assayed for RNA polymerase under standard conditions.

ratio of totalpolymerase I activity to total polymerase II activity from rat liver was about 1.5, and this ratio from AH-130 cells was 4-5. Consequently, in AH-130 cells polymerase I activity was relatively higher than in rat liver.

DISCUSSION

When RNA polymerase was solubilized from AH-130 cell nuclei by sonication in 0.3 M $(NH_4)_2SO_4$, the enzyme activity was remarkably reduced. Keller and Goor (17) showed that RNA polymerase could be solubilized at high yield from African green monkey liver by incubating a crude nuclear suspension for 5-15 hours in an isotonic buffered sucrose solution. RNA polymerase from Novikoff ascites tumor nuclei (9) was solubilized by homogenizing the crude nuclear pellet in 0.3 M $(NH_4)_2SO_4$ solution with a yield of 90%. These procedures do not involve sonication of nuclear fraction in high ionic strength, and in the present study RNA polymerase was solubilized by these procedures. After solubilization of RNA polymerase from AH-130 cell nuclei, 10% of polymerase activity remained in the residual chromatin pellet, and the possibility of selective extraction of one type of polymerase was negligible. When RNA polymerase of normal rat liver nuclei was solubilized by salt extraction without sonication, the recovery of the enzyme was at the same rate as the sonic procedure. It was concluded that in AH-130 cells, RNA polymerase I activity was higher than that of polymerase II activity. This may result from increased enzyme synthesis or an increase in the turnover of activating proteins of RNA polymerase I, as in estrogen-stimulation of r-RNA synthesis (13). The optimal concentration of $(NH_4)_2SO_4$ for RNA polymerase from AH-130 cells was similar to that for rat liver enzyme but the template requirement was altered by the ionic strength. These results may suggest that polymerase I transcribes nicked positions of template DNA more effectively than polymerase II and that polymerase II requires a specific factor for transcribing native DNA (18).

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