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SOLUBLE RNA POLYMERASE FROM RAT LIVER NUCLEI

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SUMMARY

RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) was extracted from rat liver nuclei in a soluble form. When the preparation was carried out on a sufficiently large scale to minimize adsorption losses, up to 80% of the activity and about 10% of the DNA of the intact nuclei were extracted. Elution of this soluble RNA polymerase from columns of DEAE-cellulose with ammonium sulfate yielded a single peak of activity which amounted to approx. 50% of the initial activity and which contained no more than 7% of the DNA of the soluble extract. The activity in this peak was completely dependent on added DNA and required the presence of Mn^{2+}. Its pH optimum was close to 8.5. Centrifugation of the RNA polymerase in a sucrose gradient yielded a single peak of activity having a sedimentation coefficient of approx. 16 S. The ratios of incorporation of the four labeled nucleoside triphosphates catalyzed by this enzyme resembled those of the calf thymus DNA used as a primer and were essentially identical to those obtained with purified *Escherichia coli* RNA polymerase under similar conditions.

INTRODUCTION

The DNA-dependent RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) of some animal cells appears to be present in a form which can be dissociated readily from DNA. The soluble enzyme has been prepared from chick embryo[1], bovine lymphosarcoma[2,3], rat testis[4] and HeLa cells[5]. However, the procedures used to obtain soluble RNA polymerase from these proliferating tissues yield only small amounts of the enzyme when applied to adult liver and other organs which are composed mainly of nondividing cells. Accordingly, most studies on this enzyme from these sources have been carried out with intact nuclei or with the "aggregate enzyme" of WEISS[6]. The enzyme in these preparations is firmly bound to DNA, forming an insoluble complex which precludes the study of its properties or the use of exogenous DNA templates.

RAMUZ *et al.*[7] described a method for obtaining soluble RNA polymerase from rat liver nuclei. However, we were able to extract only about 5 to 8% of the activity

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of intact nuclei in a soluble form using this procedure[8]. SEIFART AND SEKERIS[9] recently described a method, employing sonication, to extract the soluble enzyme from rat liver nuclei. The yield of enzyme obtained from the nuclei was not stated. We reported a procedure for the extraction of DNA-dependent RNA polymerase from the same source which gave high yields of the soluble enzyme. Optimal conditions were determined in terms of four extraction parameters (pH, temperature, buffer concentration and time) which yielded approx. 50% of the activity of intact nuclei in a soluble form[8]. We now report a partial purification and some properties of the enzyme extracted in this manner.

MATERIALS AND METHODS

Materials. All [^{14}C]ribonucleoside triphosphates were purchased from Schwarz BioReaserch Inc.; nonlabeled ribonucleoside triphosphates were obtained from P–L Biochemicals, Inc. Pyruvate kinase was purchased from Calbiochem. DEAE-cellulose (DE-23) was a product of W. and R. Ralston, Ltd. Female albino rats were purchased from Sprague–Dawley. Phosphoenolpyruvate and calf thymus DNA were obtained from Sigma Chemical Company. *Escherichia coli* RNA polymerase was a gift of Dr. E. Brody. Ethidium bromide (2,7-diamino-9-phenylphenanthridine-10-ethyl bromide) was a gift of Boots Pure Drug Company, Nottingham, Great Britain.

Purification of DNA. Commercially supplied calf thymus DNA was further purified by extraction with phenol at least 3 times to remove residual ribonuclease activity. The purified DNA was dissolved in 0.01 M Tris–chloride (pH 7.5) and extensively dialyzed against the same buffer containing 1.0 mM EDTA. After subsequent dialysis against 0.01 M Tris–chloride (pH 7.5), the DNA was precipitated with ethanol and dissolved in the latter buffer to a concentration of 1.0 mg/ml.

Preparation of soluble RNA polymerase. All operations, unless noted, were carried out at 0 to 4°. Nuclei were prepared using hypertonic sucrose as previously described[10] from 50 g of rat liver. The nuclei were washed with 240 ml of 0.32 M sucrose containing 1.0 mM $MgCl_2$ and subsequently with 240 ml of 0.25 M sucrose. They were then lysed by gentle homogenization (Potter–Elvehjem homogenizer with a teflon pestle) in 34 ml of 15 mM Tris–phosphate (pH 8.8) containing 0.5 mM EDTA and 1.0 mM 2-mercaptoethanol. The viscous mixture was incubated at 28 to 30° for 50 min with constant, gentle stirring. After this extraction, 15 ml of glycerol were added and the lysate was homogenized at low speed. Centrifugation of this preparation at 115 000 × *g* for 40 min yielded a clear supernatant solution which contained the RNA polymerase activity. This fraction will be referred to as the soluble extract.

Assay of RNA polymerase activity. These determinations were carried out in duplicate at pH 8.1 in a final volume of 0.57 ml containing the following: 0.02 μmole of one [^{14}C]nucleoside triphosphate (specific activity of 8 μC/μmole), 0.3 μmole of each of the other three nucleoside triphosphates, 50 μmoles of Tris, 35 μmoles KCl, 3 μmoles NaF, 25 μmoles 2-mercaptoethanol, 1.5 μmoles $MnCl_2$, 1.0 μmole trisodium phosphoenolpyruvate, 50 μg pyruvate kinase, 100 μg calf thymus DNA and enzyme. After incubation for 7 min at 37°, the reaction was stopped with 2.0 ml of 0.5 M $HClO_4$. The precipitates were collected on Millipore filters (pore size, 0.45 μ) which had been saturated with 1 mM ATP. Each filter was washed 8 times with 2-ml portions of 0.5 M $HClO_4$, dissolved in 15 ml BRAY's solution[11] and assayed for radioactivity in a

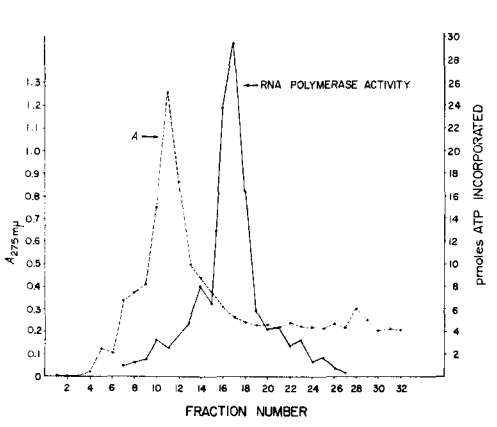
Packard Tri-Carb liquid scintillation counter. Zero-time and blank (*minus* enzyme) assays gave 60 to 80 counts/min (less than 0.5 pmole of labeled nucleoside triphosphate).

Other procedures. DNA of nuclei and lysate fractions was measured by the diphenylamine method[12]; DNA of fractions containing no particulate material was measured by the ethidium bromide fluorescence technique[13]. Calf thymus DNA was used as a standard. Protein was measured by the method of LOWRY *et al.*[14] using bovine serum albumin as a standard. Dialysis tubing was washed with 5 mM EDTA at 90° and subsequently with water before use. DEAE-cellulose was precycled before use with 0.5 M HCl and 0.5 M NaOH according to the instructions provided by the supplier.

RESULTS AND DISCUSSION

Extraction of the enzyme. The yield of soluble RNA polymerase was affected by the scale at which the extraction was carried out. When the enzyme was prepared from 5 g of liver, the yield obtained was about 50% (ref. 8). The use of 50 g of liver resulted in yields up to 80%. This increase was most likely the result of decreased losses through adsorption to glassware. Other factors which could affect the yield were considered in the preliminary account of this work[8].

Partial purification and concentration of the enzyme. The RNA polymerase activity of freshly prepared soluble extract was partially purified by DEAE-cellulose chromatography as described in the legend to Fig. 1. The use of soluble extract which had been prepared for 1 day or longer significantly reduced the amount of activity eluted from the column, even though the extract itself lost very little activity during



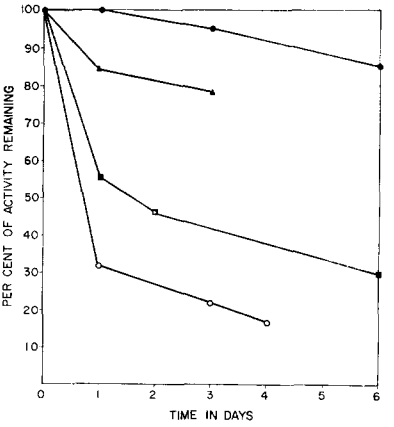


Fig. 1. DEAE-cellulose chromatography of the rat liver RNA polymerase. Between 30 and 40 ml of freshly prepared soluble extract (containing about 40 mg protein and 2 mg DNA) were applied to a column (40 cm × 1 cm) of DEAE-cellulose in the sulfate form which had been equilibrated previously with 30% glycerol, 15 mM Tris–phosphate (pH 7.7), 1.0 mM 2-mercaptoethanol. The RNA polymerase was eluted with a 0 to 0.8 M $(NH_4)_2SO_4$ linear gradient containing 30% glycerol, 15 mM Tris–phosphate (pH 7.7), 1.0 mM 2-mercaptoethanol.

Fig. 2. Stabilities of various fractions of the soluble RNA polymerase. The fractions were: ●, soluble extract; ▲, soluble extract to which $(NH_4)_2SO_4$ was added to a concentration of 0.5 M at 0 days; ■, concentrated enzyme; and ○, pooled fractions after DEAE-cellulose chromatography.

TABLE I

PURIFICATION OF RNA POLYMERASE FROM RAT LIVER NUCLEI

Source of enzyme	*Specific activity (pmoles [^{14}C]ATP incorporated/ mg DNA)*	*Specific activity (pmoles [^{14}C]ATP incorporated/ mg protein)*	*Purification in terms of DNA*	*Purification in terms of protein*	*Yield of activity (%)*	*Yield of protein (%)*
Nuclei	750	110	1	1	100	100
Soluble extract	5 600	220	7.5	2	80	40
Pooled fractions after DEAE-cellulose chromatography	40 300	710	45	6.4	40	6

that time. As shown in Fig. 1, the activity was eluted as a single peak (at about 0.5 M $(NH_4)_2SO_4$) and was separated from the bulk of material absorbing at 275 mμ. Approx. 50% of the RNA polymerase activity applied to the column was recovered in Fractions 16 through 19. These combined fractions (20 ml) contained less than 7 μg DNA/ml which represented approx. 7% of the DNA in the extract applied to the column and less than 1% of the DNA of the intact nuclei. The ratio of the absorbance at 280 mμ to that at 260 mμ for these combined fractions was 0.876. Since the protein concentration was 0.26 mg/ml, the total nucleic acid concentration was estimated to be about 14 μg/ml. A summary of the purification is shown in Table I.

The activity in the peak fractions was routinely concentrated by addition of crystalline bovine serum albumin to 1 mg/ml followed by slow addition of crystalline $(NH_4)_2SO_4$ to about 90% saturation. After approx. 12 h, the precipitate was collected by centrifugation at 12 000 $\times$ *g* for 30 min and dissolved in a small volume (about 1 to 2 ml) of 30% glycerol, 15 mM Tris–phosphate (pH 7.7), 1.0 mM 2-mercaptoethanol. Any insoluble residue was removed by centrifugation.

Stability of the enzyme. The data in Fig. 2 depict the stabilities of various fractions of the soluble RNA polymerase. The soluble extract was relatively stable; 45% of the activity remained after 8 weeks of storage at 2°. In contrast, the activity in the combined peak fractions after DEAE-cellulose chromatography was much less stable. It was not significantly stabilized by adding various combinations of the following materials to the final concentrations stated: DNA, 100 μg/ml; $MnCl_2$, 3 mM; and the four nucleoside triphosphates, 0.5 mM each. Concentration of the preparation as described above rendered some stability to the activity. This was possibly the result of the decreased concentration of $(NH_4)_2SO_4$ in this fraction; the addition of $(NH_4)_2SO_4$ to the soluble extract to a concentration of 0.5 M caused some decrease in its stability (Fig. 2).

Time course of the reaction. As shown in Fig. 3, the rate of incorporation of [^{14}C]ATP by the enzyme was nearly linear for 21 min. After 56 min of incubation, significant incorporation still occurred.

Dependency of the activity on added DNA. The RNA polymerase activity of the soluble extract contained 10 to 12% of the DNA of the intact nuclei; the incorporation of [^{14}C]ATP in the absence of added calf thymus DNA was 5 to 10% of that

incorporated in its presence[8]. Although the combined peak fractions after DEAE-cellulose chromatography contained about 7% of the DNA of the soluble extract, the RNA polymerase in these combined fractions and in the concentrated fraction was completely dependent on added DNA for activity.

Sedimentation properties of the enzyme. The upper panel in Fig. 4 shows that a single peak of activity with a sedimentation coefficient of about 15 S was obtained upon centrifugation of the rat liver RNA polymerase in a 5 to 20% linear sucrose

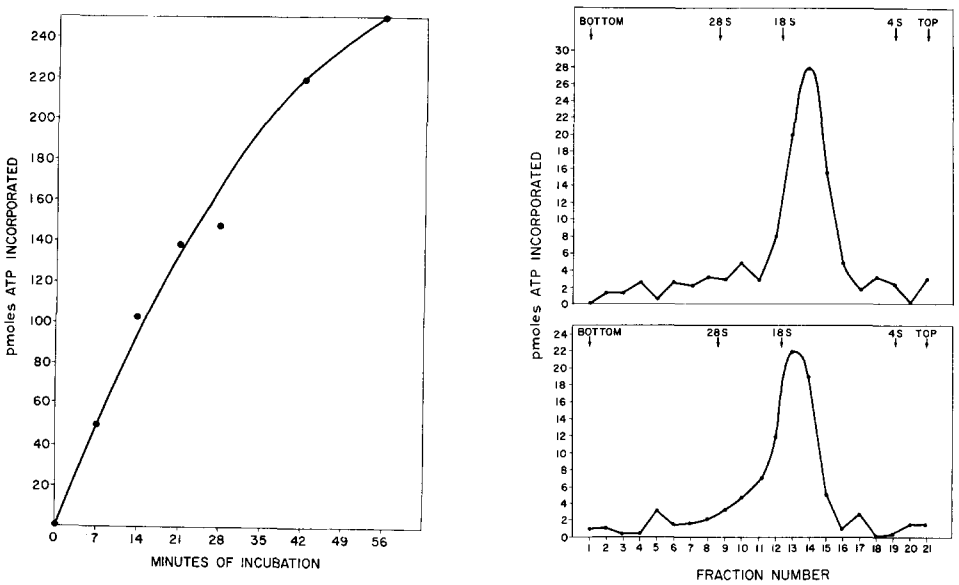


Fig. 3. Dependence of [^{14}C]ATP incorporation on the time of incubation. Duplicate assays for RNA polymerase activity were carried out as described in MATERIALS AND METHODS. Incubations were for the times noted in the figure.

Fig. 4. Sedimentation properties of the RNA polymerase. 75 μl of the concentrated enzyme fraction (containing no glycerol) were layered on 5 to 20% linear sucrose gradients (4.5 ml) containing 15 mM Tris–phosphate (pH 7.7), 0.01 M NaCl, and 1.0 mM 2-mercaptoethanol. The marker sedimentation coefficients were obtained from separate gradients on which 75 μl of purified rat liver RNA (mg/ml) were layered. The tubes were centrifuged at 45 000 rev./min in a Spinco SW50 rotor for 150 min at 2°.

gradient. The lower panel shows a duplicate experiment run 24 h later from which a sedimentation coefficient of about 17 S was estimated for the activity. Although the difference between these values is not significant, it appears from the shape of the peak in the lower panel that some association of the enzyme may have occurred after 24 h of storage at 2°. The soluble RNA polymerase of HeLa cells was sedimented in a sucrose gradient and it gave rise to a peak of activity at about 10 S and another peak at about 20 S (ref. 5).

These results serve primarily to confirm the soluble state of the enzyme. The sedimentation properties of purified *E. coli* RNA polymerase are influenced by ionic strength[15], oligodeoxynucleotides, storage in Tris buffer[16], and perhaps other unidentified factors.

TABLE II

RATIOS OF INCORPORATION OF THE FOUR NUCLEOSIDE TRIPHOSPHATES CATALYZED BY RAT LIVER RNA POLYMERASE AND *E. coli* RNA POLYMERASE

The incorporation of each nucleoside triphosphate was determined by duplicate assays as described in MATERIALS AND METHODS except that the time of incubation was 15 min.

Nucleotide incorporated	*Ratios of incorporation*		*Ratios of complementary bases in calf thymus DNA*
	E. coli enzyme	*Rat liver enzyme*	
ATP	0.256	0.235	0.283
CTP	0.281	0.282	0.214
UTP	0.261	0.268	0.286
GTP	0.202	0.215	0.217

Fidelity of transcription of DNA. The data in Table II show that the ratios of incorporation of the four labeled nucleoside triphosphates catalyzed by this enzyme were similar although not identical to the ratios of complimentary bases in the calf thymus DNA used as a primer. In an effort to identify the source of this discrepancy between the experimental and theoretical values, ratios of incorporation using purified *E. coli* RNA polymerase were measured under similar conditions. As can be seen from Table II, these ratios were essentially identical to those obtained with the rat liver enzyme. This result indicated that the deviations from the theoretical value were

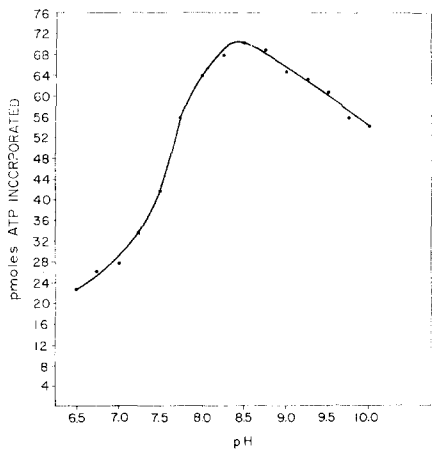


Fig. 5. Dependence of [^{14}C]ATP incorporation on pH. Assays were carried out as described in MATERIALS AND METHODS at the pH values noted in the figure. Sodium acetate buffers were used for assays at pH 6.5 through pH 7.0; Tris–chloride buffers were employed for assays at pH 7.0 through 10.0. The rate of incorporation of [^{14}C]ATP at pH 7.0 was the same for each buffer. The final buffer concentration was 0.1 M and the time of incubation was 15 min.

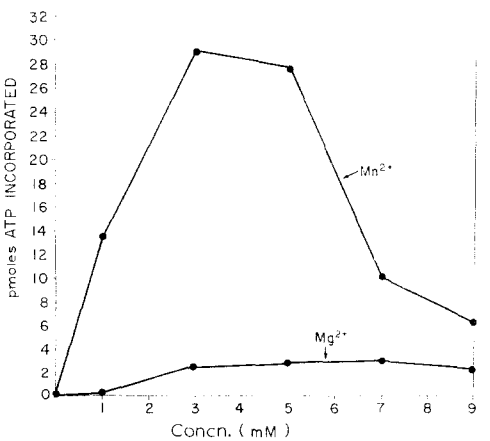


Fig. 6. Dependence of [^{14}C]ATP incorporation on the concentrations of $MnCl_2$ and $MgCl_2$. The assays were carried out as described in MATERIALS AND METHODS except that the concentrations of these divalent ions were varied as shown in the figure. The time of incubation was 15 min.

caused by one or more of the assay conditions and probably were not the result of anomalous properties of either enzyme.

pH optimum and metal ion requirement. As shown in Fig. 5, the optimum pH for synthesis was close to 8.5. Fig. 6 shows that the activity was dependent on Mn^{2+}. Other soluble RNA polymerases respond in a similar manner to Mn^{2+}. However, in the presence of optimum Mg^{2+} concentrations (5 to 8 mM), they are 35 to 50% as active as in the presence of the optimum Mn^{2+} concentration of 3 mM (refs. 3, 4, 17, 18). The response of the soluble rat liver RNA polymerase to Mn^{2+} and Mg^{2+} was quite similar to the response of ribohomopolymer formation to these ions catalyzed by *Micrococcus lysodeikticus* RNA polymerase[17] and by soluble rat testis RNA polymerase[4]. However, the soluble rat liver RNA polymerase did not catalyze extensive ribohomopolymer formation in the presence of the four nucleoside triphosphates as indicated by a nearest-neighbor analysis using [α-^{32}P]ATP as the labeled precursor as well as by the data in Table II.

CONCLUSIONS

This soluble RNA polymerase, like those described from other sources, catalyzed the incorporation of nucleoside triphosphates into RNA which reflected the base composition of the primer DNA. Its very low activity in the presence of Mg^{2+} was the only property measured which was significantly different from the other RNA polymerases. The reason for this difference is not known.

The mechanisms by which RNA synthesis is regulated in animal cells are presently unknown, although it is well documented that a variety of stimuli such as partial hepatectomy and certain hormones lead to increases in the rate of synthesis of RNA by liver nuclei. In order to evaluate the mechanisms by which these increases are brought about, studies on isolated components of the RNA synthesizing complex of nuclei obtained from animals in various physiological states would seem to be relevant.

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