BBA 63360

Partial purification of RNA polymerase from rat liver nuclei

For a number of studies, a highly purified mammalian DNA-dependent RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) would be extremely desirable, but none is available, although several workers[1-6] have made crude, soluble preparations of this enzyme. We wish to report an approx. 2000-fold purification of RNA polymerase from rat liver nuclei and to describe some of its properties. An abstract of some of this work has been published[7].

Proteins were assayed as described by BENNETT[8]. All steps of the enzyme purification procedure were carried out at 0–5°. At all stages of purification, the enzyme lost activity very slowly when stored at − 70°.

Nuclei were prepared from rat livers by the method of BLOBEL AND POTTER[9], scaled up when appropriate, and with the addition of 0.0015 M $CaCl_2$ to these investigators' isolation media.

The nuclei from 125 g of tissue were suspended in a total volume of 25 ml, using Medium S (0.05 M Tris–HCl, pH 7.8; 30% glycerol, by vol.; 0.01 M thioglycerol). The suspension was treated in a Branson Sonifier (power setting 6) in 10-sec bursts, with 15-sec cooling periods between bursts. The sonication was continued until roughly 90% of the nuclei were disrupted (90 sec). Nuclear disruption was confirmed microscopically. The nuclear sonicate was centrifuged at 78 500 × *g* for 45 min, and the sediment discarded.

Streptomycin sulfate, 1 mg for every mg of protein in the supernatant solution, was dissolved in a volume of water equivalent to one-twentieth that of the concentrated supernatant solution and was added with stirring to the supernatant solution. The streptomycin-treated solution was centrifuged at 78 500 × *g* for 30 min, and the precipitate discarded. The supernatant solution was immediately placed on a DEAE-cellulose column (in a typical preparation, 789 mg of protein were placed on a 4.4 cm × 40 cm column). The column had been previously well washed with Medium S containing 0.1 M ammonium sulfate. After the protein was adsorbed, the column was washed with 1.2 bed vol. of Medium S containing 0.15 M ammonium sulfate. Finally the enzyme was eluted with Medium S containing 0.25 M ammonium sulfate. The enzyme emerged with the front of the eluting medium.

The enzyme solution eluted from the DEAE-cellulose column was concentrated to at least 4 mg protein/ml (Diaflo pressure cell and membrane) and placed on a cellulose–phosphate column previously washed with Medium S. In a typical preparation, 20 mg of protein were placed on a 2.2 cm × 22 cm column. The column was eluted first with 1.5 bed vol. of Medium S containing 0.4 M ammonium sulfate and then with Medium S containing 0.6 M ammonium sulfate. The enzyme began to emerge after 1.1 bed vol. of this medium had passed into the column.

The purification procedure described (Table I) yields a mammalian DNA-dependent RNA polymerase with a specific activity 2000-fold higher than that of the soluble enzyme obtained from the sonicated nuclei.

The requirements for activity of the enzyme are shown in Table II. For activity, the enzyme needs all four ribonucleotides, DNA (native or denaturated), and either Mn^{2+} or Mg^{2+}. There is a sharp optimal concentration for Mn^{2+} at 3 mM, while

TABLE I

RESULTS OF PURIFICATION PROCEDURE

RNA polymerase activity was assayed as shown in a volume of 0.5 ml containing 200 mM Tris–HCl (pH 7.8), 50 mM thioglycerol, 3 mM $MnCl_2$, 2 mM each of ATP and GTP, 0.3 mM CTP, 0.5 mM [^{3}H]UTP and 130 μg DNA. The mixture was incubated 15 min at 37°. After the incubation was completed, 0.5 ml of a cold solution containing 10% trichloroacetic acid, 0.04 M sodium pyrophosphate and 0.4 mg/ml of finely dispersed bovine serum albumin was added. Then 5 ml of a solution containing 5% trichloroacetic acid and 0.02 M sodium pyrophosphate were added, and the mixture was kept on ice for 10 min. The precipitate was collected on glass fiber pads, washed thoroughly with the dilute trichloroacetic acid–pyrophosphate solution, and the radioactivity was measured in a liquid scintillation counter. In this assay, enzyme activity was linearly proportional to time (up to 15 min) and to protein concentration. 1 punit equals the incorporation of 1 pmole of UTP into RNA per min under the assay conditions. Fractions 1, 2 and 3 are, respectively, the first quarter, the middle half and the last quarter of the peak of enzyme activity eluted from the DEAE-cellulose column.

Purification step	*Total punits*	*Specific activity (punits/μg protein)*
Soluble fraction of nuclear sonicate	13 200	0.021
Streptomycin treatment	25 600	0.029
DEAE-cellulose chromatography		
Fraction 1	3 500	0.27
Fraction 2	6 000	0.96
Fraction 3	2 700	0.6
Phosphate–cellulose chromatography of Fraction 2 above	1 100	47

activity continues to rise with increasing concentration of Mg^{2+}. Optimum activity occurs at pH 7.8. These results agree with those of BALLARD AND WILLIAMS-ASHMAN[2], who studied a relatively crude RNA polymerase from rat testis. Our crude enzyme preparations were stimulated by ammonium sulfate (as are the testicular and bacterial enzymes), but were strongly inhibited by ammonium sulfate when purified.

TABLE II

CONDITIONS FOR THE RNA POLYMERASE REACTION

The assay was carried out as described in the text using 5 μg of eluate 2 from the DEAE-cellulose column.

Assay mixture	*pmoles [^{3}H]UTP incorporated into RNA per 15 min*
Complete assay mixture	72
− ATP	1
− GTP	9
− CTP	5
− DNA (native)	4
− DNA (native) + DNA (heat-denaturated)	70
− Mn^{2+}	24
− Mn^{2+}, *plus* Mg^{2+} (0.008 M)	45
− Thioglycerol	51
+ Ammonium sulfate (0.012 M)	42

This work was supported in part by U.S. Public Health Service Grant CA-03341-10.

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Received August 6th, 1968