ISOLATION AND PURIFICATION OF DNA-DEPENDENT RNA POLYMERASE FROM NUCLEI OF HUMAN PLACENTA

(NUCLEOSIDE TRIPHOSPHATE: RIBONUCLEIC ACID NUCLEOTIDYL TRANSFERASE, E.C.2.7.7.6.)

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It has been found possible to solubilise completely all the RNA polymerase activity of nuclei isolated from human placental tissue by incubating these nuclei in the presence of 120 mM $(NH_4)_2SO_4$ (AS). Following purification by chromatography on DEAE-cellulose it has been demonstrated that the soluble RNA polymerase is only active in the presence of added DNA. The final Fraction V of this preparation was nearly 100 fold enriched.

METHODS

Nuclei were prepared from the fresh placenta of normal births using Triton X-100 (cf.HYMER and KUFF,1964). Complete data on this new method will be reported elsewhere (MERTELSMANN,1968). Isolated nuclei (Fraction II, Table 2) were incubated first for 15 minutes at 37°C in buffer A (= 10 mM Tris-HCl pH 7.8 - 10 mM AS (Merck, Biochemical Grade) - 6 mM $MgCl_2$ - 1 mM K-EDTA - 5 mM β-mercaptoethanol - 30 % (V/V) glycerol - adjusted to pH 7.4 with 2 M Tris). 30 µl of AS (satured at 0°C) were added per ml of nuclei suspended in this buffer. After a second incubation for 15 minutes at 37°C with AS the suspension was centrifuged for 20 minutes at 40,000 g. The supernatant (Fraction III, S-40) showed 80-120 % of the RNA polymerase activity of the nuclei suspension (Table 2). 55 ml of Fraction III containing 12 mg/ml of protein were added to a column of DEAE-cellulose (1.8 cm x 22 cm). The DEAE-cellulose (0.64 meq./g) had previously been equilibrated with 20 volumes of buffer B (= buffer A, brought to 100 mM AS and adjusted to pH 7.4 with 2 M Tris). After the enzyme had been applied, the column was washed through with buffer B until the absorbance at 280 mµ of the effluent was less than 0.05 O.D.. Elution was carried out using a linear gradient formed by mixing 120 ml of buffer B with 120 ml of buffer C (= buffer A, brought to 500 mM AS, adjusted to pH 7.4 with 2 M Tris). RNA polymerase activity which was dependent upon the addition of exogenous DNA was eluted at a concentration of 300 mM AS. The DNA-dependent fractions were combined (Fraction IV, Table 2). The enzyme

from Fraction IV was precipitated by adding crystalline AS to a final saturation of 55 %. After centrifugation for 60 minutes at 40,000 g the precipitate was dissolved in buffer A containing 50 % glycerol.
The RNA polymerase activity was measured by the incorporation of ^{3}H- or ^{14}C-labelled nucleotides into polynucleotides. Standard assay conditions are shown in Table 1. Human DNA, obtained from placental nuclei by the method of KAY (1964), salmon sperm and calf thymus DNA (Worthington) were used as exogenous sources of DNA.

Table 1

THE STANDARD ASSAY FOR DNA-DEPENDENT RNA POLYMERASE

Tris-acetate buffer pH 9.0	250 mM
$MgCl_2$	13 mM
ß-mercaptoethanol	5 mM
ATP, CTP, GTP each	1 mM
^{3}H-UTP (specific activity = 1,300 C/mole)	40 µM
Calf thymus DNA	100 µg/ml
Enzyme protein from Fraction I, II, III (Table 2)	2500-7500 µg/ml
Enzyme protein from Fraction IV, V (Table 2)	100-500 µg/ml

50 µl enzyme solution were added; final reaction volume was 100 µl. Fractions I, II and III were usually incubated for 5 minutes at 37°C in the presence of 400 mM AS and fractions IV and V (Table 2) were incubated for 1 hour at 37°C without added AS.

Polynucleotides and nucleotides were separated by chromatography on Whatman 3MM paper (MATTHAEI et al.,1966). The solvent used for chromatography was 1 volume of 1 M ammonium acetate added to 1 volume of 95 % ethanol and adjusted to pH 4.2 by adding glacial acetic acid (modified from THACH and DOTY, 1965). Polynucleotides which remained at the starting point were counted in a Tricarb liquid scintillation counter (Packard). The counting efficiency was 55 % for ^{14}C and 3 % for ^{3}H. RNAase activity was measured by the breakdown of ^{14}C-labelled poly(U,G). DNA concentration was measured using the diphenylamine method of GILES and MYERS (1965). Protein concentration was determined by the method of LOWRY et al. (1951).

RESULTS

The product formed by Fraction IV enzyme preparation was RNAase labile and resistent against treatment with DNAase.

Table 2

ISOLATION OF DNA-DEPENDENT RNA POLYMERASE FROM HUMAN PLACENTA

	Fraction	Total protein (mg)	Total units (μU)+	Specific activity (μU/mg protein)	Fold purification
I	Homogenate	16,800	13,400	0.8	1
II	Isolated nuclei	1,070	25,000	24	30
III	S-40	830	32,000	39	49
IV	Column eluate (300 mM AS)	13	1,100	22(112)++	28(140)++
V	Ammoniumsulphate precipitate	3.2	224	70	88

+ 1 μU = 1 mμmole ^{3}H-UTP incorporated/minute at 37°C in a 100 μl standard assay

++ most active fractions of the column eluate

Moreover, the incorporation of nucleotides by the RNA polymerase was strongly inhibited by actinomycin D (Fig.1).

The more highly purified Fraction V enzyme preparation could be frozen in liquid nitrogen without loss of activity. However, Fraction IV enzyme preparation was very labile and lost all its activity within a few days at 0°C. Freezing of this fraction caused a loss of 30 % of its activity.

Fraction III enzyme preparation had similar properties to those of the isolated nuclei. The RNA polymerase activity of both fractions was greatly stimulated by AS with an optimum stimulation at an ionic strength of 1.3 (cf.WIDNELL and TATA,1966).

RNA polymerase which was eluted at 300 mM AS (Fraction IV) catalyzed the incorporation of nucleotides only in the presence of added DNA (Fig.2). Correspondingly, there was no detectable amount of DNA present in this fraction. It was also free of RNAase. Denatured DNA had a much smaller template activity compared to native DNA.

RNA polymerase activity was also dependent on the presence of all four nucleoside triphosphates. ADP, GDP and CDP could not substitute for ATP, GTP and CTP. The purified enzyme did not catalyze a DNA- or poly A-dependent synthesis of poly U.

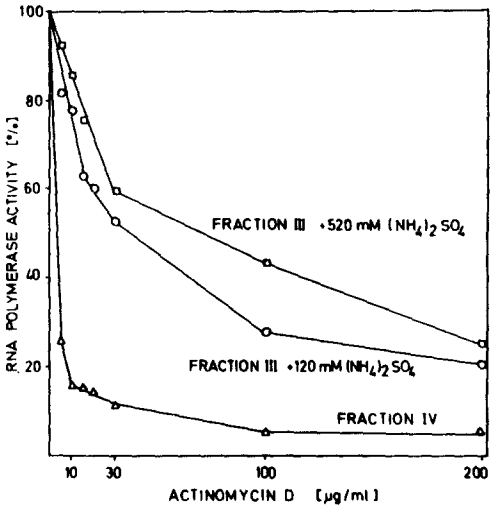

Fig.1. Inhibition of RNA polymerase activity of Fractions III and IV by various concentrations of actinomycin D (standard assay, see Table 1).

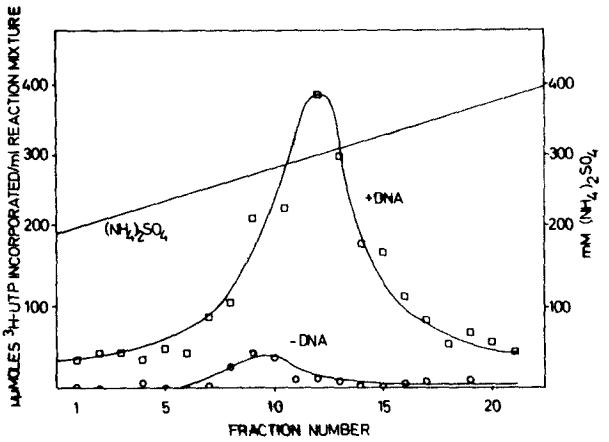

Fig.2. The DNA-dependence of the RNA polymerase of Fraction IV (standard assay, see Table 1).

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