A RAPID PURIFICATION METHOD FOR DNA-DEPENDENT RNA POLYMERASE B FROM RAT LIVER

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1. Introduction

During the regulation of gene expression the modification of the chromatin template and the activities of the RNA polymerase seem to be involved in a precise cooperation. For transcription studies it might be therefore appropriate to derive template and enzymes from the same organisms. Furthermore the application of homologous RNA polymerases avoids uncertainties and possible artefacts arising from transcription experiments with chromatin and *Escherichia coli* polymerase [1–3]. Therefore the isolation of RNA polymerase B from eucaryotes has been described repeatedly. With rat livers these procedures start with large quantities (400–1500 g liver) in order to obtain sufficient enzyme [4–6]. In the following we want to describe a purification method which starts with relatively small amounts (30–300 g rat livers) and within two days gives good yields of the enzyme in 3 simple steps.

2. Materials and methods

2.1. *Heparin–Sepharose*

Sepharose 4 B suspension (Pharmacia, Sweden) 150 ml, was activated with 12.5 mg/ml cyanogen bromide as in [7]. Then 400 mg heparin (sodium salt, Serva, Heidelberg) in 150 ml 0.1 N $NaHCO_3$, pH 8.5 were shaken at room temperature overnight with the activated gel suspension pre-washed with 0.1 N $NaHCO_3$ and 1 N NaCl, at pH 8.5. Remaining activated centres were destroyed with 1 M ethanolamine [8]. The heparin content was determined in washed and lyophilized aliquots by the method in [9] to be 12.7 μg heparin/mg Sepharose.

2.2. *Buffers*

TGMED: 50 mM Tris, pH 7.9; 30% glycerol; 5 mM $MgCl_2$; 0.1 mM EDTA; 0.5 mM dithioerythritol with ammonium sulfate molarities as indicated within brackets. Dithioerythritol was added to the buffers directly before use.

Ionic strengths were determined by measuring the electric conductivities using calibration curves.

2.3. *Polymerase B*

All operations were carried out at 0–4°C. The nuclei were obtained from livers of female Wistar rats (100–200 g) as in [10].

The nuclei from 5 livers each were sonicated [11] in 12 ml TGMED (0.3) for 5–8 s with a Branson sonifier (Model S 75, microtip, setting 4, 3 A) and the ionic strength reduced by addition of 20 ml TGMED (0). The resulting suspension was vigorously stirred for 15 min, then centrifuged at 80 000 × g for 60 min.

To the clear supernatants 7% (w/v) polyethyleneglycol 4000 (PEG) (Serva, Heidelberg) was added and 10 min after solubilization centrifuged again 60 min. By addition of powdered PEG the concentration was brought to 25% (w/v) and the precipitated protein centrifuged as above. The sediment was dissolved in a small volume of TGMED (0.3) and applied to the heparin–Sepharose column (3.9 mg protein/ml Sepharose) equilibrated with TGMED (0.3). The column then was washed with 1.5 column vol. same buffer and eluted with 1.5 vol. TGMED (0.5). The fractions containing enzyme activity were pooled and

dialyzed against TGMED (0) to 0.1 M ammonium sulfate. Subsequently the solution was given on a column with DEAE–Sephadex A-25 (Pharmacia, Sweden) equilibrated with TGMED (0.1). After washing with TGMED (0.1) the enzyme was eluted with 2 column vol. TGMED (0.5).

2.4. *Enzyme assay*

The reaction mixture consisted of 50 mM *N*-2'-hydroxypiperazine-2-ethanesulfonic acid (Serva, Heidelberg), pH 8.1, 150 mM KCl, 1 mM $MnCl_2$, 5 mM $MgAc_2$, 12 mM mercaptoethanol, 100 μg/ml calf thymus DNA, 5 μCi/ml [^{3}H]UTP and 0.6 mM ATP, CTP, GTP and 0.06 mM UTP. The reaction was started with 30 μl enzyme solution and the samples (150 μl final vol.) were incubated at 37°C for 10 min. The reaction was terminated by addition of 10% trichloroacetic acid + 20 mM sodium-pyrophosphate. The precipitates were collected on Nitrocellulose-filters (Sartorius, Göttingen) and washed with 30 ml 5% trichloroacetic acid and dried for 30 min at 60°C. The radioactivity on the filters was measured in a Packard Tri-Carb Scintillation Counter with a dioxan-Scintillator. By addition of α-amanitin (2–150 μg/ml). the RNA polymerases could be assigned.

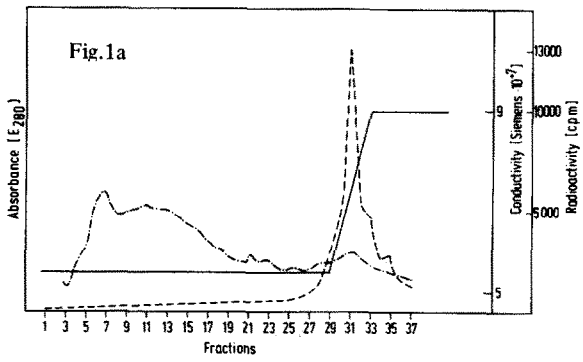
2.5. *Electrophoreses*

These were performed as in [12] under non-denaturating condition in 4% acrylamide gels or by the Laemmli method [13] in 10% SDS–gels. Protein was determined by the Lowry method [14].

3. Results and discussion

The described isolation method for RNA polymerase B is a 3 step procedure: after selective precipitation with polyethyleneglycol follow column chromatographies on heparin–Sepharose and subsequently DEAE-Sephadex. In comparison to [4–6,15,16] the above-described method can be performed very rapidly and needs less animals.

The duration of sonication was kept as short as possible because a loss of enzyme activity was observed with time. A precipitation with ammonium sulfate led to inconsistent results [16] in comparison to the fractionation with polyethylene glycol (PEG) which yields the enzyme activity in a concentrated form also removing the nucleic acids [17,18]. The precipitation with 25% PEG cannot be ommitted because the 7% PEG supernatant showed impaired flow and fractionation properties of the subsequent heparin–Sepharose column. It should be mentioned that in the 25% PEG precipitate after solubilization only part of the enzyme activity can be measured presumably because of a template compaction by PEG [19]. Chromatography on heparin–Sepharose already has been applied successfully in RNA polymerase isolation [20–25]. By equilibrating the column at relatively high ionic strength we aproached ion filtration conditions and strong interactions of the enzyme with the column material during chromatography were avoided [26]. Most of the contaminating proteins are separated first, fig.1a. Finally the chromatography on DEAE–Sephadex serves both as a purification and concentration step, fig.1b. On both columns the gradient elution shows no advantages in



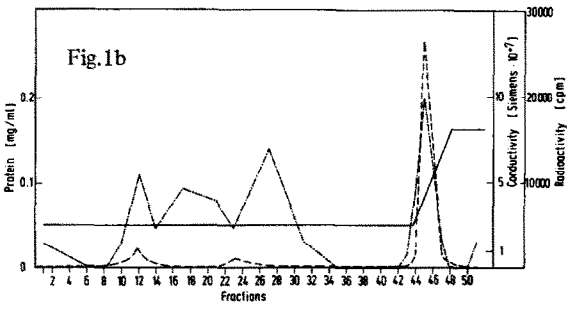


Fig.1. Elution profiles on: (a) heparin–Sepharose; (b) DEAE–Sephadex. (–·–·) protein (E_{280}), (——) enzyme activity (cpm) incorporated [^{3}H]UTP (see assay), (——) electric conductivity of the fractions.

Table 1
Yields and increase of enzyme activity during the purification of RNA polymerase B from rat livers

Fraction	Total enzyme (units)	Specific activity (units/mg protein)	Purification (factor)	Recovery (%)
80 000 × *g* supernatant	386	1.3	1	100
7% PEG supernatant	365	1.3	1	95
Heparin–Sepharose eluate	210	15	11	54
DEAE eluate	192	210	161	50[a]

[a] 75% with 200 g rats

The activities sensitive to 2 μg/ml α-amanitin are listed. 1 enzyme unit corresponds to the incorporation of 1 nmol [^{3}H]UTP/10 min at 37°C

comparison to step elution; on the contrary the enzyme would be isolated in a dilute and more labile form [27].

Stabilization of the enzyme activity with bovine serum albumin was avoided, since this might influence subsequent transcriptional studies. The purification procedure was performed with 30–300 g rat livers but may be easily extended to larger quantities. With 200 g rats the overall yield was 75%, with 100 g rats only 50%, however, because of higher enzyme levels in the young animals the total yield was better for this group, table 1. The enzyme purity was asserted

Fig.2a

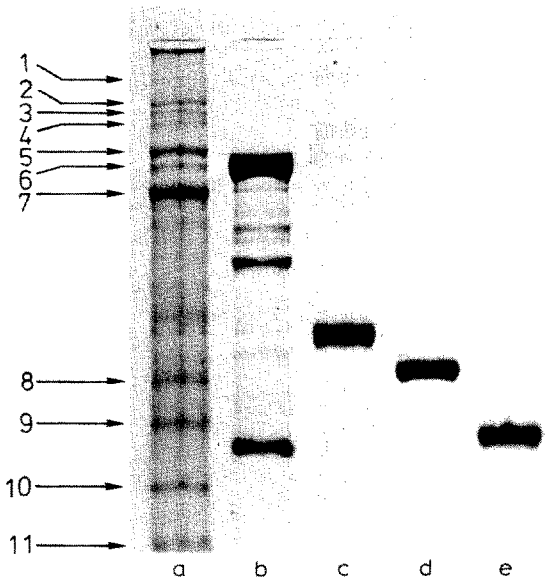


Fig.2a. SDS–acrylamide gel electrophoresis of: (a) Purified polymerase B; (b) *E. coli* polymerase ($\beta'\beta\delta\alpha$); (c) catalase (CAT); (d) ovalbumin (OV); (e) bovine serum albumin (BSA).

Fig.2b

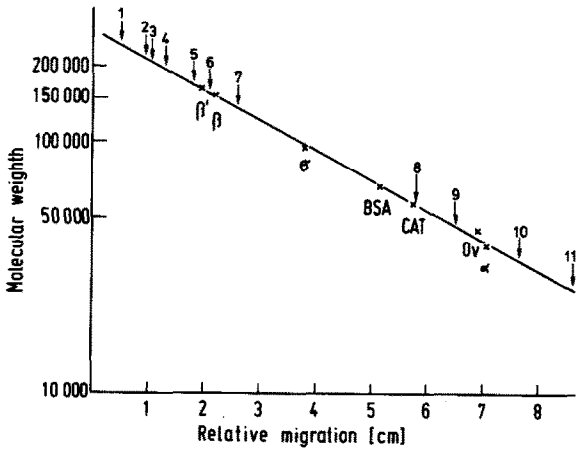


Fig.2b. Electrophoretic mobilities on SDS gels and molecular weight correlations. The band pattern (240), 215, 210, 195, 170, 158, 137, 57, 47, 34, 27.5 (kD) was found to coincide with the enzymatic activity. (The 240 kD band only can be observed after prolonged electrophoresis. Under normal conditions additional bands with 25, 20, 15, 14 and 11.5 kD can be observed.) Bands 1–11 correspond to the numbering in fig.2a.

by the following criteria: coincidence of enzyme activity with the protein peak after DEAE chromatography; unchanged SDS subunit pattern over the activity containing fractions; and by electrophoreses under non-denaturating conditions in which two bands appear. In agreement 2 or 3 forms of rat liver polymerase B have been reported [4–6,28]. In comparison a larger number of high and low molecular weight bands are found during SDS electrophoresis, fig.2a,b. Our observation of 6 or 7 high molecular weight bands may be the consequence of the rapid isolation procedure which minimizes proteolytic degradation of large subunits, a process which has been shown to occur in several tissues [4,21,29–31].

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