MOLECULAR WEIGHT AND SUBUNIT STRUCTURE OF RNA POLYMERASE I AND INITIATION FACTOR FROM CHROMATIN OF PLANT CELL NUCLEI*

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Received 16 May 1973

1. Introduction

Multiple forms of RNA polymerase activity have recently been reported from eukaryotes [1]. The molecular basis of this heterogeneity is at present unknown but, whatever its basis, the crucial question seems to be whether different enzymes copy different genes. A common feature of all these enzymes is that they differ from each other in response to ionic and template requirements, sensitivity towards α-amanitin, subunits and in nuclear and nucleolar localization. We have recently described the isolation and purification of enzymes and factors from chromatin of plant cell nuclei [2,3]. This paper describes the first attempt to purify the initiation factor for RNA polymerases from the chromatin of plant cell nuclei and determination of the molecular weight and subunit nature of both the enzyme and factor as revealed by SDS polyacrylamide gel electrophoresis.

2. Materials and methods

2.1. *Materials*

Green coconuts (*Cocos nucifera*) 4—5 months old were obtained from local market. ATP, GTP, UTP, CTP and ion-exchange resins were purchased from Sigma Chemical Company (St. Louis, Mo., USA). Aquacide was purchased from CalBiochem (Löwengraben, Switzerland). Acrylamide, *N,N'*-methylene bisacrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED) were all purchased from Eastman Kodak Company, Rochester, N.Y., USA. Coomassie Brilliant Blue R 250 (CoLab), Amido black (G.T. Gurr) and Fast green (E. Merck) were commercial products.

2.2. *Preparation of chromatin*

Nuclei were isolated from coconut endosperm by the method of Mondal et al. [4] in the presence of 0.25 M sucrose in 0.01 M Tris–HCl, pH 8.0. The crude chromatin was isolated from the nuclei by the method of Bonner and Huang [5]. Details of the procedure are described elsewhere [2].

2.3. *Preparation of enzymes and factor*

RNA polymerase was isolated from the nonhistone protein according to the method of Mondal et al. [2]. RNA polymerase I and II were obtained after chromatography on DEAE cellulose. The peak eluted at 0.5 M KCl on DEAE column had a stimulatory role on both the enzymes. Both purified enzymes and factor were stored at −12°C in a buffered medium containing 50% glycerol.

2.4. *Assay of RNA polymerase activity*

The reaction mixture containing 40 mM Tris–HCl, pH 8.0, 0.2 mM EDTA, 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$ or 2 mM $MnCl_2$, 0.4 mM K_2HPO_4, 0.16 mM NaCl, 0.1 mM of each of the four triphosphates of which one was labelled, [^{3}H]UTP, specific activity 4 cpm/pmol, 40 μg/ml coconut endosperm DNA and RNA polymerase (RC I) (100 μg) and factor B (10 μg) in a total volume of 250 μl. After 15 min incubation at 37°C, RNA synthesis was measured as incorporated in

* Part IV of the series "RNA polymerase from eukaryotic cells".

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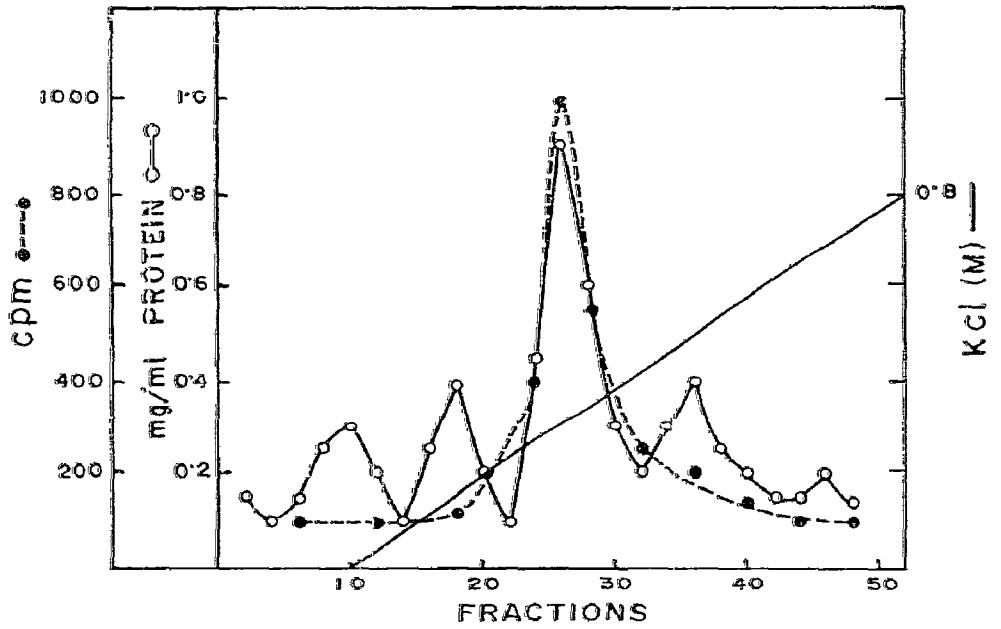


Fig. 1. QAE–Sephadex (A-50) chromatography of factor B from chromatin. The protein preparation in 0.01 M Tris–HCl buffer, pH 8.0 containing 1 mM M.E., 0.1 mM EDTA and 5% v/v glycerol was passed through a QAE–Sephadex column, precycled and equilibrated with the same buffer. The column was washed and then eluted with a 0–0.8 M KCl linear gradient in the above mentioned buffer. Fractions of 2 ml were collected. Aliquots containing 5 μg of protein were assayed with 50 μg of QAE enzyme (RC I) as described earlier [2]. (•—•—•) Factor B activity; (o—o—o) protein, mg/ml; (——), M KCl.

TCA precipitable material and counted on filter paper discs as previously described [2].

Protein was determined according to Lowry et al. [6].

2.5. *Electrophoresis of the native enzyme at high pH*

The purified enzyme RC I and factor B (5 μg each) was layered on polyacrylamide gels and subjected to electrophoresis (3 mA/gel at 4°C) for 90 min [2]. These conditions presumably maintain the protein molecule in its native state.

The subunit structure of the enzyme and factor were analyzed by the SDS polyacrylamide gel electrophoresis. The purified enzyme and factor B (20 μg each) were separately incubated at 37°C for 3 hr in 0.1 M sodium phosphate (pH 7.1), 0.1% SDS and 0.1% 2-β mercaptoethanol. The incubated proteins were layered on polyacrylamide gels (5%) and subjected to electrophoresis (10 mA/tube at 37°C) for 3 hr [7].

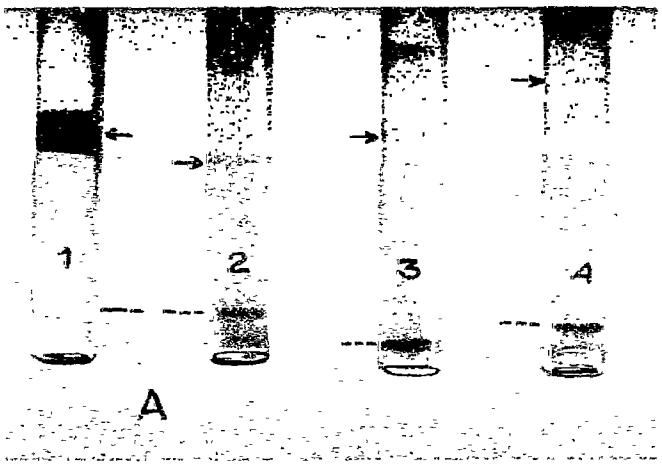
3. Results

3.1. *Further purification of factor B*

The protein peak eluted from DEAE cellulose column at 0.5 M KCl (fraction B) had a stimulatory activity on both the RNA polymerases. This fraction was further subjected to QAE–Sephadex (A-50) column chromatography and was eluted with a linear gradient of 0–0.8 M KCl (fig. 1). The fractions were assayed for stimulating RNA polymerase I and the active fractions were pooled and concentrated by aquacide.

3.2. *Homogeneity of RNA polymerase I and factor B*

Dialyzed samples of RC I and factor B were analyzed in native polyacrylamide gels at different pH and with different gel concentrations using different dyes to test the homogeneity. Acrylamide at the concentrations of 3%, 3.5% and 5% was used in case of RC I, whereas, 7%, 7.5% and 8% was used in case of factor B. Coomassie Brilliant Blue R 250, Amido



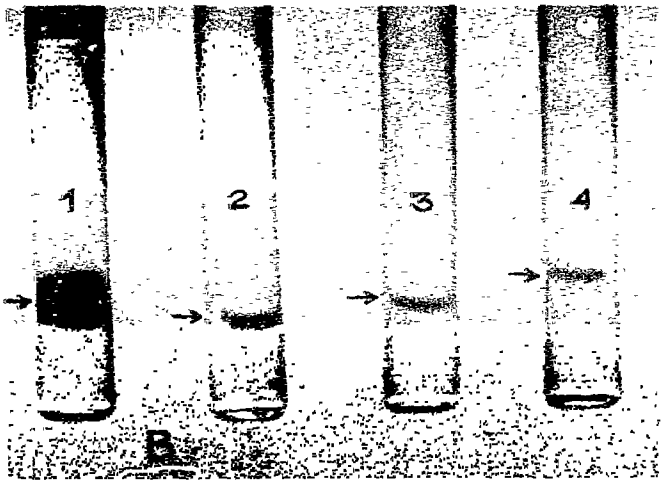


Fig. 2. Polyacrylamide gel electrophoresis of purified RNA polymerase I and factor B. Detailed procedure is described under Materials and methods. A) 1, 50 μg RC I in 3% acrylamide; 2, 5 μg RC I in 3% acrylamide; 3, 5 μg RC I in 3.5% acrylamide; 4, 5 μg RC I in 5% acrylamide. B) 1, 50 μg factor B in 7% acrylamide; 2, 10 μg factor B in 7% acrylamide; 3, 10 μg factor B in 7.5% acrylamide; 4, 10 μg factor B in 8% acrylamide. The gels were electrophoresed at pH 8.4 and were stained with Coomassie Brilliant Blue. Arrows indicate the protein bands and the dotted lines mark the dye front.

black and Fast green were used for staining where Coomassie blue gave maximal sensitivity. The results are depicted in fig. 2. From fig. 2 A, it can be seen

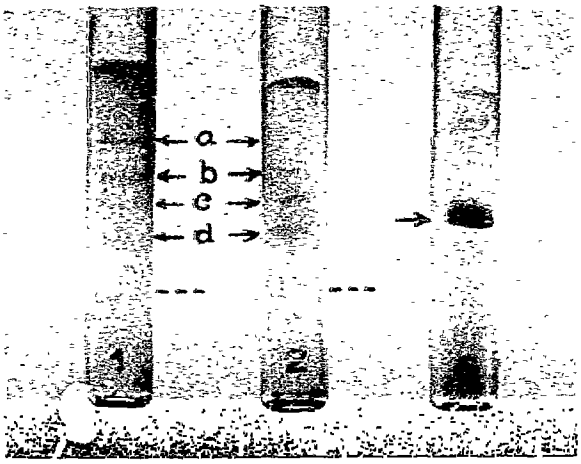


Fig. 3. Polyacrylamide gel electrophoresis of RC I and factor B in presence of SDS. 1) 20 μg RC I in 5% acrylamide, stained with Coomassie Brilliant Blue. 2) Same as in 1, stained with Amido black. 3) 20 μg of factor B in 5% acrylamide, stained with Coomassie Brilliant Blue. Dotted line indicates dye front.

that even on loading 50 μg of the enzyme RC I, a single band was obtained. Also, only one band was obtained in three different gel concentrations. The same results were obtained in the case of factor B (fig. 2 B) indicating that both the enzyme and factor were homogeneous proteins. Also, both the enzyme and factor B gave single bands when run in single gels (the spacer and sample gels omitted) in buffers of pH 6.4, 7.4 and 8.4.

3.3. *Subunit nature of enzyme and factor*

The subunit structures of purified RNA polymerase (RC I) and factor B were analyzed by the SDS polyacrylamide disc gel electrophoresis. By means of this technique and densitometric tracing of the gels stained by Coomassie Brilliant Blue R 250 and Amido black, the constituent subunits were identified, their molecular weights were estimated, and the subunit compositions were determined.

Four subunits were resolved after subjecting the RNA polymerase RC I to SDS polyacrylamide gel (fig. 3). The molecular weights of the subunits of RC I a, b, c and d (determined from a standard curve of molecular weight vs. the distance of migration from the origin of pure proteins of known molecular weight) were found to be 175 000, 150 000, 95 000 and 80 000, respectively. All these four subunits in the same molar ratio were found even if the QAE—

Sephadex purified RC I has been passed through Biogel P 300 column before gel electrophoretic analysis, which suggests that the four subunits are always associated with the enzyme activity. The subunit structural formula for purified RC I was thus assigned as ab_2cd_2 with a molecular weight of 730 000. The purified fraction B (initiation factor) gave single band even in 0.1% SDS–polyacrylamide gel and the molecular weight of approx. 76 000 was obtained.

4. Discussion

RNA polymerase from *E. coli* contains four subunits besides σ and ρ factors [8], whereas the present system contains at least six polypeptide chains and the molecular weight of this preparation (RC I) has been shown to be around 730 000 compared to 400 000 of *E. coli* enzyme. The molecular weight of the enzyme from the rat liver has recently been reported to be around 750 000 [9] and 500 000 in the case of thymus enzyme [10] and 440 000 for the yeast enzyme [11]. In general, the complexity in the structure of RNA polymerase in eukaryotes seems to be increased by the additions of more subunits to it. The interconversion of one form to the other has also been suggested [9]. In that case, some of the subunits might act as initiation or other factors. However, smaller subunits as reported in the case of mammalian RNA polymerase [12] have not been detected in the present case.

Practically nothing is known about initiation or termination factors in eukaryotic cells. That the fraction B acts as an initiation factor in the case of plant RNA polymerase has been proved from several lines of evidence [3]. The molecular weight of this initiation factor has now been found to be 76 000. At optimal concentration of this initiation factor several fold increase in RNA synthesis by RNA polymerase I has been recorded and this was obtained only when the ratio of RNA polymerase I to initiation factor was 10:1 [3]. From this stoichiometry and the molecular weight it is suggestive that one molecule of RNA polymerase I interacts with one molecule of initiation factor to form the initiation complex with DNA. The complex of RNA polymerase I and factor B has been found to be resistant to rifampicin.

Acknowledgement

This work was supported by research grant from USDA PL-480 fund (FG-In-321).

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