A NEW METHOD FOR THE PURIFICATION OF RNA-POLYMERASE

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Received February 2, 1967

Several methods for the isolation and purification of RNA-polymerase (Furth et al, I962 ; Chamberlin and Berg, I962 ; Nakamoto et al, I964 ; Ochoa et al, I96I ; Richardson, I966 ; Stevens, Henry, I964 ; Fuchs et al, I964) from microorganisms have been described in the literature. With the exception of the method of Fuchs (Fuchs et al, I964), all procedures employ either protamine or streptomycin or both for the initial steps of purification. This paper describes a method which avoids the use of these compounds and gives high yields of enzyme. The method uses, in addition to ammonium sulfate precipitation and DEAE Chromatography, the principle of phase partition (Albertson, I960), which has been used by Okasaki and Kornberg (Okasaki, and Kornberg, I964), in order to separate nucleic acids from the DNA-polymerase of B. subtilis. We have found that the use of successive phase separations permits not only the separation of RNA-polymerase from nucleic acids, but also a substantial and highly reproducible increase in the specific activity of the enzyme.

MATERIALS AND METHODS

Unlabeled nucleoside triphosphates were purchased from

P-L-Biochemicals Laboratories, $ATP^{8\text{-}CI4}$ from Schwarz Bioresearch Inc. Polyethyleneglycol 6000 (PEG 6000) was purchased from Union Carbide Inc. and Dextrane 500 from Pharmacia, Uppsala. Thymus DNA was prepared by the method of Kay et al (I952).

The bacterial strain used for the extraction of the RNA-polymerase was the RNAase$^-$AI9 (Gesteland, I966), kindly provided by Dr. J. Richardson.

RNA-polymerase activity was measured according to Furth et al (I962), except that only Mn^{++} was added to the reaction mixture, which had a final volume of 250µl. After IO min. incubation at 37°C, the reaction was stopped by the addition of 4 ml of cold 5 % TCA. The precipitate was then collected on a membrane filter (Millipore filter HA 25, 0.45 µ) and washed with 5 ml cold 5 % TCA followed by 5 ml cold 0.5 % TCA. The filter was then dried and the radioactivity incorporated as AMP-C^{I4} (I.700 cpm/mµm) counted in a Packard Tri-Carb scintillation counter.

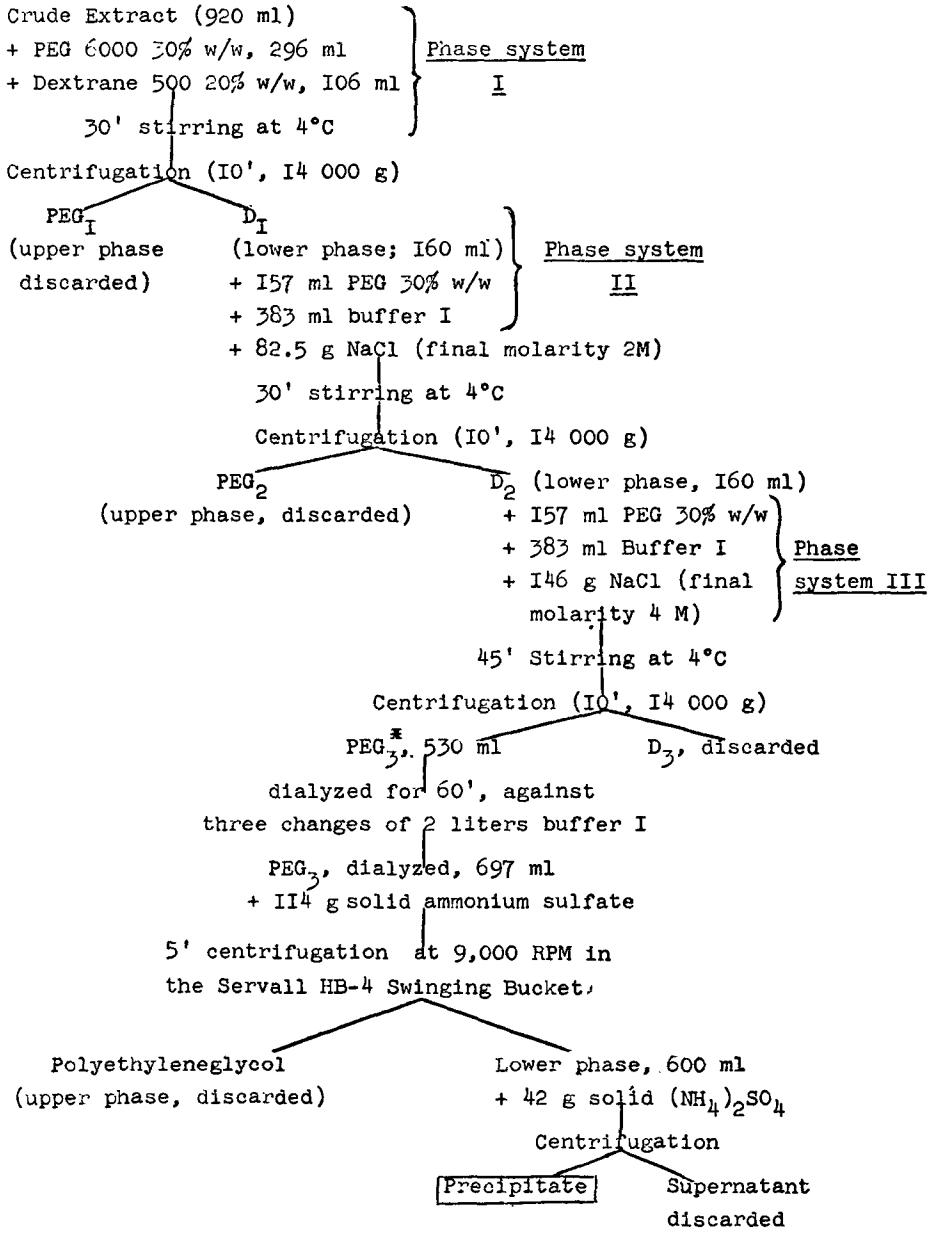
Protein was measured by the procedure of Lowry (Lowry et al, I95I) or by the optical density at 280 mµ, (0.66 OD_{280}/ mg / ml) as indicated.

RESULTS - PURIFICATION OF THE ENZYME

I. - Preparation of "ASI"

In a typical preparation 250 g of frozen A I9 cells were ground for 20 min. with 500 g of alumina. The mixture was then suspended in I liter of buffer I (0. 0I. M Tris HCl pH 8.4, 0.005 M 2-mercaptoethanol, 0.0I M Mg Cl_2 0.00I M EDTA) and centrifuged for 30' at 9,000 RPM in a

Servall RC2 centrifuge. The supernatant (Crude extract) was then treated according to the procedure given in the diagram. (figure I).



* The PEG_3 solution was dialyzed with sufficient agitation to keep it homogenious within the dialysis-bag.

- Figure I -

The final precipitate was dissolved in 40 ml Buffer II (0.0I M Tris HCl pH. 8.4, 0.005 2-mercaptoethanol, 0.00I M EDTA). This solution (ASI) contained 80 % of the initial activity.

2. - DEAE - Sephadex chromatography

A column of DEAE Sephadex (I8 cm x 5 cm2) was prepared and equilabrated with buffer II. Fraction ASI, was dialyzed for 60 min. against two changes of 4 liters buffer II, and then diluted to a final volume of I20 ml with the same buffer to give a final protein concentration of 5 mg / ml, before being adsorbed to the column at a rate of I ml / min. The column was then washed with 50 ml buffer II, and eluted in the following manner : a) the column was washed with buffer I containing 0.25 M KCl and 50 ml fractions were collected until the OD_{280} was below 0.05/ml., b) the process was repeated with buffer II containing 0.33 M KCl, and the activity was finally eluted by buffer II containing 0.40 M KCl. The fractions containing the activity were combined (I50 ml) and 44 g of solid ammonium sulfate were added to the protein solution. The precipitate was collected by centrifugation and dissolved in 35 ml of a buffer containing 0.05 M Tris HCl pH. 8.0, 0.005 M 2 mercaptoethanol, 0.00I M EDTA. This fraction (AS 2) contains 57 % of the initial activity.

3. - Properties of the purified enzyme

The different steps of the purification are summarized in Table I. The overall recovery in the preparation described here was 57 %, and the specific activity of AS 2 was I400 units / mg of protein as measured by the method of

Lowry (Lowry et al, I95I). The ratio of adsorbance at 280 mμ to that at 260 mμ was I.6. The enzyme was stable for two weeks at 4°C ; it was stored in liquid nitrogen, and did not loose any activity under these conditions for several monthes. Using the data of Richardson (I966), (specific absorbance of enzyme = 0.65 OD/mg/ml at 280 mμ), 90 mg of pure enzyme were obtained from 250 g of frozen bacteria. The sedimentation pattern, at high ionic strength (0.5 M KCl), in the analytical ultracentrifuge reveals two peaks, one of which is symetrical and sediments with an S value of I3.7 S, and a minor peak representing not more than 5 % of the total material. No DNAase activity could be detected as measured by the test of Lehman et al (Stevens, Henry, I964), using I00 μg of fraction AS 2 in the 30' assay. The preparation was judged free of RNAase activity as measured by the lack of hydrolysis of polyribocytidylic acid during a 24 hrs. incubation at room temperature. (L. Hirschbein, unpublished procedure.

Table I

	Total Units	% Recovery	Specific activity units/mg prot.
Crude Extract	I80.000	I00	I0
PEG_3 dialyzed	I65.000	9I	-
ASI	I56.000	85	250
AS2	I05.000	57	I400

ACKNOWLEDGEMENTS

This work has been supported by grants from the National Science Foundation, the "Délégation Générale à la Recherche

Scientifique et Technique", the "Commissariat à l'Energie Atomique", and the "EURATOM".

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