Short Communications

A Procedure for the Rapid Purification of *Escherichia coli* Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase

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(Received 29 January 1973)

A rapid procedure involving DNA–cellulose chromatography followed either by sedimentation in a high-salt glycerol gradient or by gel filtration is described for the complete purification of *Escherichia coli* DNA-dependent RNA polymerase.

A very simple method for the complete purification of *Escherichia coli* DNA-dependent RNA polymerase (EC 2.7.7.6) involving a minimum of time and effort is described. After treatment with polyethylene glycol to remove debris and nucleic acids, the crude extract is chromatographed on DNA–cellulose by using

methods similar to those developed by Alberts *et al.* (1968) and Litman (1968) for the purification of proteins with affinity for DNA. The enzyme is finally purified either on a high-salt glycerol gradient or by high-salt gel filtration and contains only the β' , β , τ , σ and α bands on polyacrylamide gels containing

Fig. 1. DNA–cellulose chromatography (a) and glycerol-gradient sedimentation (b) of RNA polymerase

(a) Experimental details are given in the text. $\circ$, E_{280} ; Δ , RNA polymerase activity (measured with calf thymus DNA and without added KCl). (b) A 2 ml portion of DNA–cellulose-purified enzyme in buffer A containing glycerol (50 g/l) and KCl (50 mmol/l) was layered on to a 13 ml 10–30% (v/v) glycerol gradient in buffer A containing KCl (1.0 mol/l). Tubes were centrifuged at 75000g (r_{av} , 10.5 cm) for 60 h at 4°C in the aluminium rotor (no. 59108) of an MSE Superspeed-75 ultracentrifuge: 21 fractions were collected dropwise from the base of each tube and assayed for RNA polymerase activity. $\circ$, E_{280} ; Δ , RNA polymerase activity, measured as in (a) above.

Fig. 2. Polyacrylamide-gel electrophoresis of RNA polymerase

Enzyme preparation (10 μ g) in a volume of 30 μ l containing sodium dodecyl sulphate (1.0 g/l) was run for 75 min at 45 V in gels containing polyacrylamide (50 g/l) and sodium dodecyl sulphate (1.0 g/l), the running buffer containing sodium phosphate, pH 7.1 (10 mmol/l), and sodium dodecyl sulphate (1.0 g/l). Gels were stained with Coomassie Brilliant Blue R-250 and scanned at 650 nm in a Gilford spectrophotometer.

sodium dodecyl sulphate. The procedure up to the gradient takes less than 1 day for 100 g lots of cells and can easily be scaled up without an equivalent increase in the work load.

DNA-cellulose was prepared from Whatman CF11 resin and double-stranded calf-thymus DNA (type V; Sigma Chemical Co., St. Louis, Mo., U.S.A.) by the method of Alberts & Herrick (1971) with the addition of irradiation steps as described by Litman (1968). RNA polymerase activity was assayed by the method of McConnell & Bonner (1972) and *E. coli* B (mid-exponential phase) was supplied by General Biochemicals, Chagrin Falls, Ohio, U.S.A. Buffer A contained Tris-HCl buffer, pH 7.9 (10 mmol/l), $MgCl_2$ (10 mmol/l) and dithiothreitol (0.1 mmol/l).

The following operations were carried out at 4°C. A 100 g portion of cells was lysed by grinding with

alumina and suspended in 180 ml of buffer A containing glycerol (50 g/l) and KCl (2.0 mol/l). The suspension was centrifuged at 10000 g for 15 min to remove debris and the supernatant was made up to 200 ml with buffer A containing glycerol (50 g/l) and KCl (2.0 mol/l). A solution containing polyethylene glycol 6000 (300 g/l) and KCl (2.0 mol/l) was slowly added to the supernatant to give a final polyethylene glycol concentration of 60 g/l. After gentle stirring for 15 min the cloudy suspension was centrifuged at 10000 g for 15 min and the supernatant was dialysed for 5 h against 10 litres of buffer A containing glycerol (50 g/l) and KCl (50 mmol/l). After centrifugation at 10000 g for 10 min to remove a fine precipitate, the supernatant was loaded on to a DNA-cellulose column (17 cm high $\times$ 5 cm diam.) at a flow rate of 250 ml/h. The column was washed with buffer A containing glycerol (50 g/l) and KCl (0.15 mol/l) until the flow-through emerged and was then developed with buffer A containing glycerol (50 g/l) and KCl (0.75 mol/l). As shown in Fig. 1(a), RNA polymerase emerged in a sharp peak, and it was precipitated by adding 2 vol. of saturated $(NH_4)_2SO_4$, pH 7.0, and resuspended in 5–10 ml of buffer A containing glycerol (50 g/l) and KCl (50 mmol/l). After dialysis for 1 h against 3 litres of buffer A containing glycerol (50 g/l) and KCl (50 mmol/l), the solution was layered on to 10–30% (v/v) glycerol gradients containing KCl (1.0 mol/l) and centrifuged as described in Fig. 1(b). Enzyme fractions were pooled as shown, precipitated with 2 vol. of saturated $(NH_4)_2SO_4$, pH 7.0, redissolved in a small volume of buffer A containing glycerol (600 g/l) and stored at $-20^\circ C$. Enzyme preparations were analysed by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis by the method of Shapiro *et al.* (1967) and contained only the β' , β , τ , σ and α bands, as shown in Fig. 2. A 100 g portion of cells yielded up to 20 mg of enzyme with activities up to 1400 units/mg [1 unit of RNA polymerase incorporates 1 nmol of GTP into RNA in 10 min in the presence of KCl (0.15 mol/l) with bacteriophage T7 DNA]. As an alternative to glycerol gradients, RNA polymerase was chromatographed on Bio-Gel A-1.5m. DNA-cellulose-purified RNA polymerase from 100 g of cells was successfully purified on a 2.7 cm $\times$ 34 cm column developed at a flow rate of 0.5 ml/min with buffer A containing glycerol (50 g/l) and KCl (1.0 mol/l). The activity was eluted immediately after the excluded volume, well ahead of the other DNA-affinity proteins.

Most RNA polymerase purification methods are derived from the procedure of Chamberlin & Berg (1962), in which the cell extract requires ultracentrifugation and selective precipitation by streptomycin, protamine sulphate and $(NH_4)_2SO_4$ before further purification by column chromatography (Nakamoto *et al.*, 1964; Frederick *et al.*, 1969; Matsukage *et al.*, 1969; Abraham *et al.*, 1972). In

addition, final enzyme preparations (fraction IV as described by Chamberlin & Berg, 1962) are usually impure (<50% enzyme). By using the present method it is possible to dispense with the numerous and time-consuming preliminary fractionations characteristic of other purification procedures and to obtain a highly purified enzyme.

This work was supported by the National Science Council of Ireland.

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