

Rapid and simple purification of T7 RNA polymerase

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There is a permanent and increasing interest in large amounts of RNAs synthesized *in vitro* by bacteriophage T7 RNA polymerase. Especially for physical studies, mg quantities of RNA are required and have in fact been synthesized (1, 2). In order to encounter the resulting demand for T7 polymerase, we have developed a simple purification scheme for this enzyme, using the expression system of Studier (3). We describe here the preparation of 90 mg highly purified T7 RNA polymerase within 3 days, starting from 8g *E. coli* BL21/pAR1219 cells. This procedure involves only one instead of three column fractionations and gives a higher yield.

In the following we proceed essentially as described by Grodberg and Dunn (4), however, with the following deviations:

(a) The protease inhibitor leupeptin was replaced by bacitracin (10 μg/ml) and benzamidine (0.1 mM).

(b) After polymin P and ammonium sulfate precipitation we used S-Sepharose FF (Pharmacia) for column fractionation instead of Trisacryl-SP. Column dimensions and protein elution (flow rate: 4 to 5 drops per min) were as described (4).

(c) The S-Sepharose FF column fractions were analysed by SDS-polyacrylamide gel electrophoresis (Fig. 1A).

(d) It is most important for the success of the following steps that only the fractions 11 to 13 with the highest protein (T7 RNA polymerase) concentration are pooled.

(e) The pooled fractions 11 to 13 (2.7 ml) were dialysed against 2×500 ml at 4°C, however, the dialysis buffer C (4) contained only 10 mM NaCl, and dialysis has to be performed overnight (>15h). The precipitate formed during dialysis is T7 RNA polymerase which is collected by centrifugation (5 min at 10,000×g), dissolved in 10 ml 100 mM NaCl in buffer C and dialysed overnight against 900 ml 100 mM NaCl in buffer C containing 50% glycerol. The resulting dialysate (3 ml, containing 90 mg T7 RNA polymerase) was stored in aliquots at −20°C. This enzyme batch was analysed by SDS-polyacrylamide gel electrophoresis (lanes a and b, Fig. 1B). The specific activity was about 400,000 U/mg. As stated by Milligan and Uhlenbeck (5), this enzyme is stable for a long time, provided that additional DTT is added every 6 months to prevent oxidative inactivation. The precipitation step can be repeated if necessary. In this case, the precipitate is dissolved in 1.5 ml 100 mM NaCl in buffer C, dialysed overnight and the new precipitate processed as above.

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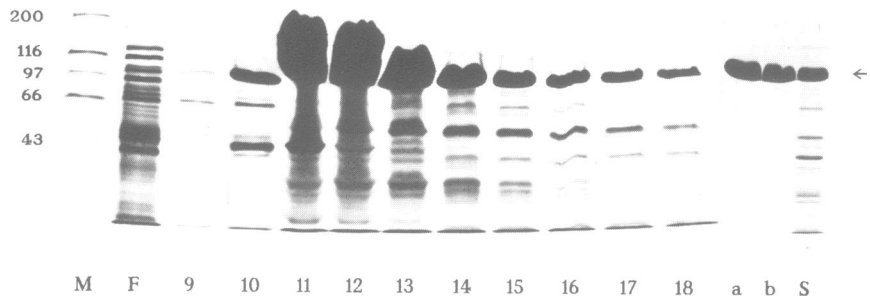

Figure 1. Analysis of T7 RNA polymerase fractions by SDS/10% polyacrylamide gel electrophoresis. M: Molecular weight markers (kDa). F to 18: S-Sepharose FF chromatography fractions. F: Flowthrough. 9 to 18: Fraction numbers. 20 μl aliquots were analysed. a: 140 μg; b: 90 μg purified T7 RNA polymerase, obtained upon precipitation during dialysis against buffer C/10 mM NaCl, collected by centrifugation, dissolved in buffer C/100 mM NaCl and dialysed against the same buffer containing 50% glycerol. S: Supernatant after dialysis against buffer C/10 mM NaCl. Staining was with Coomassie Brilliant Blue R-250.
The arrow indicates the position of T7 RNA polymerase.