

Identification of a component separated on Mono Q purification of *Escherichia coli* RNA polymerase as an NTPase

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Standard preparations of *Escherichia coli* RNA polymerase (RNAP) contain NTPase activity. High-performance anion-exchange chromatography on Mono Q has recently been used by Hager et al. [1990, Biochemistry 29, 7890–7894] to fractionate RNAP into holoenzyme ($\alpha\beta\beta'\sigma$) and core ($\alpha\beta\beta'$) forms, plus other protein components. We found that one of these components, of protomer size slightly larger than the σ^{70} subunit, has NTPase activity; it is efficiently separated on Mono Q, leaving transcriptionally active holoenzyme and core apparently free of NTPase activity. Because of the similarity in size with σ^{70} , the NTPase component may escape detection by routine gel electrophoresis.

RNA polymerase; NTPase (nucleoside 5' triphosphatase)

1. INTRODUCTION

RNA polymerase from *Escherichia coli* (RNAP) as routinely prepared [1,2] appears to be nearly homogeneous, in the sense that preparations contain very low levels of protein components that cannot be assigned to the standard subunits. However, such RNAP does contain NTPase activity. Furthermore, there is evidence that an NTPase activity associated with RNAP could function in assuring transcriptional fidelity [3–5]. To attempt to remove NTPase activity we used high performance anion-exchange chromatography on Mono Q, recently shown by Hager et al. [6] to fractionate RNAP into holoenzyme ($\alpha\beta\beta'\sigma$) and core ($\alpha\beta\beta'$) forms, RNAP subassemblies and other protein components. We show here that on Mono Q an NTPase activity is efficiently removed as a component similar in size to the σ^{70} subunit, representing an unidentified component detected by Hager et al., leaving RNAP holoenzyme and core apparently free of NTPase activity.

2. MATERIALS AND METHODS

RNAP was prepared according to Burgess and Jendrisak [1] with the modification of Lowe et al. [2]. Free σ subunit was prepared according to Lowe et al. [2] from such RNAP preparations, followed by chromatography on Mono Q (as indicated below: σ eluted at approximately 440 mM NaCl). [γ - 32 P]ATP was from NEN Dupont. TGED buffer contains 10 mM Tris buffer, pH 7.9, 5% glycerol, 100 μ M ethylenediamine tetraacetate, 100 μ M dithiothreitol.

A prepacked Mono Q 5/5 column (Pharmacia) was operated essentially as described by Hager et al. [6] using a Pharmacia FPLC instru-

ment. 3 mg RNAP diluted to 1 mg/ml in TGED + 250 mM NaCl were adsorbed onto a 5/5 Mono Q column equilibrated in TGED + 300 mM NaCl, then a gradient of 300 to 500 mM NaCl in TGED run in 120 min at 0.5 ml/min.

Gel electrophoresis in sodium dodecyl sulfate (SDS)-containing polyacrylamide was performed in 0.5 mm gels, using the Laemmli [7] system, with an upper stacking gel of 4% acrylamide.

NTPase activity was measured with [γ - 32 P]ATP by scintillation counting of the released [32 P]-orthophosphate, after adsorption of unreacted ATP on charcoal (as in [8]). Conditions: 1.2 mM ATP, 40 mM Tris buffer, pH 8, 4 mM $MgCl_2$, 200 mM NaCl, 37°C for 90 min. ADP was essentially the exclusive nucleotide product, verified by thin layer chromatography (see [5]).

Transcriptional activity of the parent RNAP and Mono Q fractions was assayed with T7-DNA according to Chamberlin et al. [9].

RNAP and σ^{70} concentrations were based on ϵ_{280} (1 mg/ml) of 0.62 for holoenzyme, 0.55 for core and 0.84 for σ^{70} [2].

3. RESULTS AND DISCUSSION

RNAP was fractionated on a Mono Q column (Fig. 1) using a very shallow NaCl gradient, as described by Hager et al. [8]. Core and holoenzyme elute in two successive peaks centered at ~390 and ~410 mM NaCl, respectively, as judged by the subunit pattern on SDS polyacrylamide gel electrophoresis (SDS-PAGE, Fig. 2a). NTPase activity (Fig. 1) elutes between 350 and 360 mM NaCl with two minor A_{280} peaks well separated from core and holo, which show no NTPase activity. The transcriptional activity of the separated, NTPase-free RNAP (measured for the holoenzyme fraction on T7-DNA) is equal to or slightly greater than that of the parent RNAP preparation. On SDS-PAGE the NTPase activity correlates with a protein component migrating at nearly the same rate as σ^{70} on 8.75% gel (commonly used for screening RNAP preparations, Fig. 2a), and slightly less rapidly than σ^{70} on 6% gel (Fig. 2b). Mono

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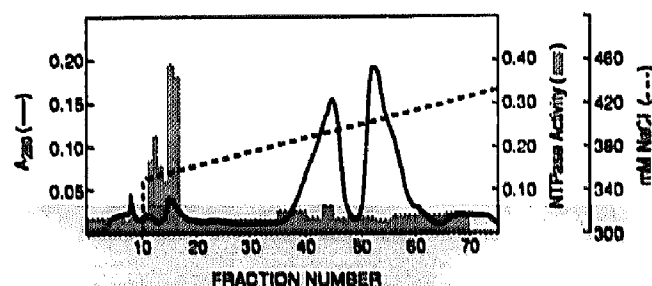


Fig. 1. Fractionation of RNAP on Mono Q. Load, 3 mg. Fraction size, 0.5 ml. Overall protein monitored by A_{280} . NTPase activity measured (at 1:1 dilution of fractions) as the proportion of counts not adsorbed by charcoal. The NTPase activity of the parent RNAP preparation was 0.09 at 300 mM RNAP.

Q fractions with higher NTPase activity have greater staining intensity in the band just above σ^{70} in the more porous gel (Fig. 2b). Mono Q fractions on the leading edge of the NTPase zone showed an additional band migrating faster than α (Fig. 2a) whose relative amount does not correlate with NTPase activity. As the closeness of the NTPase and σ^{70} bands in Fig. 2b indicates, the NTPase component may easily escape detection by routine gel electrophoresis. Although the NTPase appears to be nearly the same size as σ^{70} , it apparently is unrelated since free σ^{70} elutes from Mono Q at NaCl concentrations greater than does holoenzyme.

This is the first demonstration of apparently complete separability of an NTPase and the polymerase protein, in which both fractions were accessible. Volloch et al. [4] earlier removed an unidentified NTPase from RNAP by affinity binding. Paetkau and Coy [8] and Ishihama et al. [10] obtained partial separations of RNAP and a protein with NTPase activity that migrated on SDS-PAGE between σ^{70} and α . Due to the different electrophoretic behavior it is unclear whether the NTPases extracted by the latter two groups, which appear to be the same, are related to the NTPase we have detected. However, in Mono Q fractionation of RNAP preparations, Hager et al. [6], just as we, found a non- σ protein eluting in the low salt range, that on SDS-PAGE

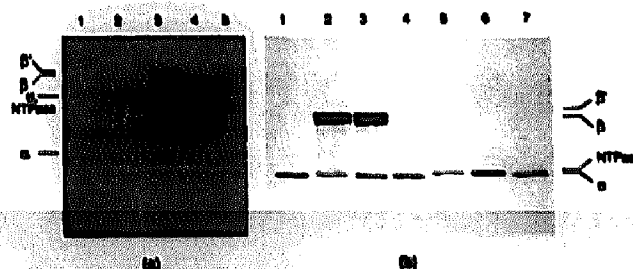


Fig. 2. SDS-PAGE of RNAP and Mono Q fractions. (a) 8.75% gel: lanes 1 and 2, fractions 11 and 16 (20 μ l); 3, fraction 43 (core, 2 μ g); 4, σ^{70} (0.5 μ g); 5, fraction 53 (holo, 2 μ g). (b) 6% gel: lanes 1, 4 and 7, σ^{70} (0.2 μ g); 2, parent RNAP (2 μ g); 3, fraction 52 (holo, 2 μ g); 5 and 6, fractions 13 and 16 (8 μ l). On gel (b), bands below σ^{70} have run off.

migrates immediately behind σ^{70} . This material very likely represents the same component as the one we separate. Thus, the RNAP purification scheme suggested by Hager et al. removes NTPase in the form of a σ^{70} -sized component.

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