Purification and Properties of a Bacteriophage T5-Modified Form of Escherichia coli RNA Polymerase

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A modified form of Escherichia coli RNA polymerase that contains one of the three T5-specific polypeptides known to interact with the host enzyme was purified from bacteriophage T5-infected cells. The properties of this T5-modified enzyme appeared identical to those of the RNA polymerase derived from uninfected non-colicinogenic cells and to a fully active enzyme isolated from T5-infected ColIb+ cells after the limited in vivo transcription of T5 genes allowed by the plasmid had ceased.

The RNA polymerase of the host cell Escherichia coli is responsible for the synthesis of the three distinct classes of bacteriophage T5-specific RNA formed during T5 infection. By immunoprecipitation studies we have shown that a class I (pre-early) T5-specific polypeptide of 11,000 daltons and two class II (early) polypeptides of 90,000 and 15,000 daltons, respectively, become associated with the RNA polymerase during the infectious cycle (C. Szabo, B. Dharmrongtartama, and R. W. Moyer, Biochemistry, in press). We have presented data to suggest that at least one of the three polypeptides, the 90,000-dalton species, tentatively referred to as a T5-specific "σ" factor, is involved in the in vivo regulation of class III (late) transcription and is likely the product of gene C2. If the host E. coli cell contains the colicinogenic factor ColIb (ColIb+ cells), T5-specific class I RNA is formed normally. However, after the initiation of class II phage RNA synthesis, transcription is arrested and the infection aborts. As expected, infected colicinogenic cells show only the pre-early T5 peptide of 11,000 daltons associated with the polymerase (C. Szabo et al., in press).

We have purified RNA polymerase from T5-infected non-colicinogenic cells 20 min after infection, using the standard procedures as described by Burgess and Travers (2). Before purification, the infected cultures had been continuously labeled with [3H]leucine under conditions that label all three classes of T5 proteins, including those we find bound to the polymerase. After each step of purification, a portion of the partially purified enzyme was immunoprecipitated with antisera prepared against the uninfected E. coli RNA polymerase holoenzyme, and the presence of the three phage-specific proteins in the immunoprecipitate was quantitated by analysis on sodium dodecyl sulfate (SDS)-polyacrylamide gels. The first step of the polymerase purification is the precipitation of the enzyme by ammonium sulfate such as we routinely used in our earlier immunoprecipitation experiments (Szabo et al., in press) and which, we have observed, has no effect on the number or amount of the phage polypeptides bound to the polymerase (data not shown). The RNA polymerase precipitated in the ammonium sulfate step is then purified by DEAE-cellulose chromatography, which removes all but the 15,000-dalton class II polypeptide from the enzyme (Fig. 1A). This remaining T5 polypeptide was rather strongly associated with the polymerase and remained bound through a subsequent purification step utilizing glycerol gradient centrifugation (Fig. 1B). After the glycerol gradient step of purification, our preparations of RNA polymerase were greater than 95% homogeneous and contained an equivalent amount of the 15,000-dalton subunit per mol of enzyme as was originally present in the immunoprecipitates prepared from crude extracts. We will refer to this purified form of RNA polymerase that contains a single T5 polypeptide of 15,000 daltons isolated from infected non-colicinogenic cells as RNAP(T5)(15), in agreement with our suggested nomenclature (Szabo et al., in press).

A sample of the RNAP(T5)(15) from cells was then analyzed on an SDS-polyacrylamide gel and the total subunit pattern was compared with that of a preparation of RNA polymerase purified from uninfected cells. Coomassie blue-
stained polypeptide bands with mobilities corresponding to the $\beta$, $\beta'$, $\sigma$, and $\alpha$ subunits of the host enzyme were detected in both preparations (Fig. 2). Since we have shown that all of the radioactivity associated with the phage polypeptide of 90,000 daltons was lost during this method of purification (Fig. 1), the stained polypeptide of 90,000 to 95,000 daltons present in the RNA polymerase purified from infected cells was probably the host $\sigma$ factor, which has a molecular weight of 95,000. From these results it appears that T5 infection does not permanently destroy the host $\sigma$ protein, and furthermore neither the association of the 15,000-dalton class II phage polypeptide nor any other T5 modification permanently prevents the host $\sigma$ factor from associating rather strongly with the enzyme. It is not known at this time whether the binding of the T5-specific "$\sigma$" factor and the host $\sigma$ factor are mutually exclusive.

We compared the in vitro transcriptional properties of RNAP(T5)(15) with those of three other purified enzyme preparations: (i) RNA polymerase from uninfected non-colicinogenic cells; (ii) RNA polymerase from uninfected ColIb$^+$ cells; and (iii) RNA polymerase obtained from T5-infected ColIb$^+$ cells that had ceased all in vivo transcription. The properties of the RNA polymerase purified from T5-infected ColIb$^+$ cells seemed particularly pertinent for this study. Although no ColIb$^+$ factor protein has been found associated with RNA polymerase (Szabo et al., in press), it is still quite conceivable that the plasmid might antagonize T5 transcription as a result of a catalytic modification of the polymerase, which might be detected by an alteration in the in vitro transcriptional properties of the enzyme. The RNA polymerase derived from each of the four preparations chromatographed on DEAE-cellulose as a single peak that eluted at 0.19 M KCl and after low-salt glycerol gradient centrifugation was at least 95% homogeneous. Fully active enzymes were recovered from all preparations including the one from infected ColIb$^+$ cells. All the purified preparations, when analyzed on SDS-polyacrylamide gels, appeared to have the host $\sigma$ subunit (Fig. 2; unpublished data). A summary of the enzymatic activities of the four

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**Fig. 1.** SDS-polyacrylamide gel electrophoresis of $^3$H-labeled T5-specific proteins bound to purified RNA polymerase. A 100-ml sample of unstarved E. coli K-12 W3110 thy$^-$ cells in T-MGM medium (6) was infected with T5st(0), a heat-stable deletion mutant of T5$, at a multiplicity of infection of 10. Leucine was then added to a final concentration of 0.1 $\mu$g/ml, and a total of 500 $\mu$Ci of [4,5-$^3$H]leucine (50 mCi/ $\mu$mol) (New England Nuclear Corp.) was added in aliquots to the infected culture at 1-min intervals from 5 to 14 min after infection. At 15 min, the $^3$H-leucine was chased with 0.2 mg of unlabeled leucine per ml, and after 20 min of infection chloramphenicol (0.1 mg/ml final) was added and the culture was harvested by centrifugation. The $^3$H-labeled cells were added to 8 g of identically grown and infected unlabeled carrier cells, and the RNA polymerase was purified by the procedure of Burgess and Travers (2) and assayed as previously described (1). After each step of purification, a portion of the RNA polymerase was immunoprecipitated as described (Szabo et al., in press). The $^3$H-labeled, precipitated samples were analyzed by SDS-polyacrylamide gel electrophoresis as described by Weber and Osborn (10). The data are presented as $^3$H counts per minute per gel slice where the $A_b$ and $A_b'$ symbols indicate the positions in the gel of the stained immunoglobulin heavy (55,000 daltons) and light chains (25,000 daltons), respectively. (A) DEAE-cellulose-purified RNA polymerase; (B) low-salt glycerol gradient-purified RNA polymerase.
After electrophoresis, the preparations purified from uninfected cells still contained the host σ factor (Fig. 2A). Although the σ subunit of RNA polymerase is required for the efficient transcription of T4 DNA, transcription proceeded quite effectively with calf thymus DNA in the complete absence of the σ subunit. Thus, the ratio of template activities with T4 and calf thymus DNA has been defined as a measure of functional σ subunit activity (8). This ratio with enzyme containing the σ subunit derived from uninfected cells was approximately 2, and the ratio did not appear to change either as a result of infection with T5 or from any effect of the ColIb plasmid (Table I). These data may be compared with those obtained for the RNA polymerase lacking the σ subunit that was isolated after T4 infection and had an activity ratio with T4 and calf thymus DNA of 0.2. Our results suggest that each of these preparations contains a purified enzyme preparation, utilizing various templates, is presented in Table 1. All four preparations yielded approximately the same total number of enzyme units (data not shown) and had similar specific activities with a given DNA template. T5 DNA was found to give the maximum in vitro activity.

SDS-polyacrylamide gel analysis of purified RNA polymerase from late T5-infected non-colicinogenic cells suggested that the enzyme still contained the host σ factor (Fig. 2A). Although the σ subunit of RNA polymerase is required for the efficient transcription of T4 DNA, transcription proceeded quite effectively with calf thymus DNA in the complete absence of the σ subunit. Thus, the ratio of template activities with T4 and calf thymus DNA has been defined as a measure of functional σ subunit activity (8). This ratio with enzyme containing the σ subunit derived from uninfected cells was approximately 2, and the ratio did not appear to change either as a result of infection with T5 or from any effect of the ColIb plasmid (Table I). These data may be compared with those obtained for the RNA polymerase lacking the σ subunit that was isolated after T4 infection and had an activity ratio with T4 and calf thymus DNA of 0.2. Our results suggest that each of these preparations contains a purified enzyme preparation, utilizing various templates, is presented in Table 1. All four preparations yielded approximately the same total number of enzyme units (data not shown) and had similar specific activities with a given DNA template. T5 DNA was found to give the maximum in vitro activity.

**Fig. 2.** SDS-polyacrylamide gel electrophoresis of RNA polymerase purified through a low-salt glycerol gradient. The preparations were electrophoresed on 7.5% SDS-polyacrylamide gels for 8 h at 8 mA per gel. After electrophoresis the protein bands were visualized with Coomassie blue. (A) RNA polymerase purified from uninfected non-colicinogenic E. coli K-12 W3110thy-; (B) RNA polymerase purified from T5-infected non-colicinogenic E. coli K-12 W3110thy- cells after 20 min of infection.

**TABLE 1.** Template activities of RNA polymerase purified from uninfected and T5-infected cells*

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Sp act (U/mg with various DNA templates</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T5*</td>
<td>T4*</td>
</tr>
<tr>
<td>Uninfected E. coli K-12</td>
<td>1,215</td>
<td>987</td>
</tr>
<tr>
<td>W3110thy*</td>
<td>965</td>
<td>749</td>
</tr>
<tr>
<td>T5-infected E. coli K-12</td>
<td>1,184</td>
<td>1,045</td>
</tr>
</tbody>
</table>

* RNA polymerase from each source was purified through the low-salt glycerol gradient step as described by Burgess and Travers (2) to greater than 90% homogeneity. The assay contained (in 0.25 ml) Tris-hydrochloride (0.04 M), MgCl2 (0.01 M), EDTA (0.1 mM), dithiothreitol (0.1 mM), KCl (0.15 M), potassium phosphate (0.4 mM), bovine serum albumin (0.5 mg/ml), undegraded DNA, UTP, GTP, CTP, ATP (0.1 mM each) (P-L Biochemicals), and RNA polymerase (2 to 5 μg per assay) at a final pH of 7.9. Each assay also contained 2 μCi of [3H]ATP (New England Nuclear Corp.). After incubation for 10 min at 37°C, the reaction was terminated by the addition of 2.5 ml of cold trichloroacetic acid (5%, wt/vol) containing sodium pyrophosphate (0.1 mM). The samples were allowed to stand on ice for 15 min and the precipitate was collected on glass fiber filters (Schleicher and Schuell no. 29), washed with a solution of trichloroacetic acid (2%, wt/vol) that contained sodium pyrophosphate (1.01 M), dried, and counted.

* 10 μg of undegraded T5 DNA was used per assay.

* 10 μg of undegraded T4 DNA was used per assay.

* 25 μg of calf thymus DNA was used per assay.
functional host factor. Additional evidence supporting this hypothesis is the effect of KCl on the in vitro enzyme activity. Host core polymerase activity (lacking the σ subunit) is only negligibly affected by KCl, whereas the holoenzyme activity is stimulated by KCl up to a concentration of 0.2 M (9). All of our preparations of RNA polymerase required 0.2 M KCl for maximum activity.

Piska and Buchanan (7) have studied the specificity of the in vitro transcription of T5 DNA by host RNA polymerase purified from uninfected cells. They found that the host σ enzyme transcribed primarily class I and class II sequences and only a small amount of class III sequences. We have shown that the net enzymatic activity of purified RNA polymerase does not appear to be altered either by the ColIb plasmid or by T5 infection. However, RNA polymerase from late T5-infected cells, under the proper conditions, may recognize and transcribe predominantly late T5 gene sequences, which are not efficiently read by the enzyme from uninfected cells. Likewise, RNA polymerase purified from T5-infected ColIb + cells possesses normal net activity, although the possibility exists that this enzyme may have lost the ability to transcribe class II T5 gene sequences. To test these possibilities, the specificity of the in vitro transcription of native T5 DNA by all four of the RNA polymerase preparations we have described was examined by hybridization-competition analysis.

3H-labeled T5 RNA that was synthesized in vitro was hybridized to T5 DNA in the presence of unlabeled T5 RNA synthesized in vivo. The competition of the labeled in vitro RNA with an excess of the in vivo RNA isolated at various times after infection measures the proportion of each class of RNA synthesized in vitro. The specificity of the in vitro transcription is thus determined, since each temporal class of in vivo T5 RNA is transcribed from a unique set of T5 gene sequences. Figure 3 illustrates the results of the hybridization-competition analyses, and the data show that all four of the enzyme preparations described above transcribe T5 DNA with the same specificity. Unlabeled

![Graph](https://via.placeholder.com/150)

**Fig. 3.** Hybridization-competition analysis of T5 RNA synthesized in vitro by different preparations of purified RNA polymerase. Labeled in vitro RNA and unlabeled in vivo RNA were purified by the hot phenol method described by Lembach and Buchanan (4). Unlabeled competitor preparations were isolated from infected cells at 3, 8, and 20 min after infection. [3H]RNA was synthesized in vitro by a slight modification of the standard RNA polymerase assay system of Burgess (1). Each mixture contained Tris-hydrochloride (0.04 M), MgCl₂ (0.01 M), EDTA (0.1 mM), dithiothreitol (0.1 mM Calbiochem), KCl (0.2 M), potassium phosphate (0.4 mM), bovine serum albumin (0.5 µg/ml), native undegraded T5 DNA (20 µg), UTP, GTP, CTP, and ATP (0.1 mM each) (P-L Biochemicals), and RNA polymerase (10 µg) in a final volume of 0.5 ml. The RNA was labeled by the addition of [5,6-3H]UTP (New England Nuclear Corp.) at a final specific activity of 125 mCi/mmol. The reaction was carried out at 37°C for 15 min before isolation of the RNA. [3H]RNA was synthesized in vitro by RNA polymerase isolated from either uninfected non-colicinogenic E. coli K-12 W3110 thy- (O), or colicinogenic E. coli K-12 W3110 thy- (Colib +) (•) and T5-infected non-colicinogenic E. coli K-12 W3110 thy- (O) or colicinogenic E. coli K-12 W3110 thy- (Colib +) cells (△). The labeled RNA was hybridized to T5 DNA that had been adsorbed to membrane filters (Millipore Corp.) (0.3 µg of DNA per filter) as described by Cooper (3). Each hybridization mixture contained [3H]RNA (10,000 counts/min), 0.3 µg of T5 DNA, and the indicated amount of unlabeled T5 in vivo competitor RNA isolated at: (A) 3 min; (B) 8 min; and (C) 20 min after infection.
phage RNA isolated 3 min after infection consisted of only class I species and was partially competitive with the [3H]RNA synthesized in vitro, indicating that at least 25% of the in vitro RNA in each case was composed of class I gene sequences (Fig. 3A). Phage RNA isolated 8 min after infection consists of both class I and class II gene sequences (5). When this RNA was used as the competitor, only 20% of the in vitro [3H]RNA remained in hybrid form (Fig. 3). Therefore, the greatest proportion of the RNA synthesized in vitro with all four enzyme preparations consisted of early sequences. All three classes of phage RNA were present in the RNA isolated 20 min after infection. Although class I RNA was not synthesized from 8 to 20 min after infection, hybridizable sequences of class I RNA were present in the cells, even though this class of RNA was no longer metabolically active. All the [3H]RNA was displaced from the hybrid by 20-min competitor RNA, indicating that all RNA species synthesized in vitro are also present in infected cells. All four enzyme preparations transcribed class I and class II sequences more efficiently than class III sequences, since only about 15% of the in vitro RNA consisted of class III sequences.

The similar catalytic properties of these four preparations fail to suggest any independent role for the T5 polypeptide of 15,000 daltons that is found associated only with the RNA polymerase purified from infected non-colicogenic cells. Furthermore, the isolation of a fully active preparation from infected ColIb* cells with properties similar to those for control preparations suggests that either any plasmid-directed inactivation of RNA polymerase activity toward T5 DNA is readily reversible or that the inactivation of transcription may be the indirect consequence of some other plasmid-directed modification of cellular properties.

(This work is taken in part from a thesis submitted by C.S. to the Faculty of Pure Science; Columbia University, in partial fulfillment of the requirements for the Ph.D. degree.)

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LITERATURE CITED