AN INHIBITOR OF HOST SIGMA-STIMULATED CORE ENZYME ACTIVITY THAT PURIFIES WITH DNA-DEPENDENT RNA POLYMERASE OF E. COLI FOLLOWING T4 PHAGE INFECTION*

Audrey Stevens

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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Summary—The content of the sigma subunit (as detected by gel electrophoresis) and activity with T4 DNA were examined with RNA polymerase fractions from both normal and T4 phage-infected E. coli. Sigma-containing fractions and core enzymes were obtained by phosphocellulose column chromatography. The sigma-containing fraction of the enzyme from infected cells, although somewhat stimulatory to both core enzymes alone, inhibits the normal sigma-stimulated activity of the core enzyme from infected cells at both low and high KCl concentration. Normal core enzyme activity is inhibited only at high KCl concentration.

The sigma subunit (σ) of E. coli RNA polymerase can be removed from the holoenzyme by phosphocellulose chromatography (1,2). The resulting core enzyme has very low activity with T4 DNA (or other phage DNA's) as a template until σ is added back (1). Sigma effects initiation of specific RNA chains and acts catalytically (3-5). With T4 DNA, the formation of early T4 mRNA requires the presence of σ (3).

The fate of σ at different stages of the T4 phage infection cycle is not clear. Much disparity exists in reported values of the σ content of RNA polymerase isolated from T4 phage-infected cells. High values have been reported for enzyme isolated shortly after infection (6,7), and values ranging from none (3,6,7) to substantial amounts (8,9) have been reported for enzyme isolated at later times. Purification techniques used may determine the amount of σ found with the enzyme. All the enzymes studied (3,6-9) had a lower activity than normal polymerase when assayed with T4 DNA. Travers (10,11) isolated from T4 phage-infected cells a protein fraction which stimulates core enzyme activity, but which differs from normal σ in effecting the synthesis of a different class of early T4 mRNA. Evidence for anti-σ activity has been reported by Bogdanova et al. (8) and Khesin et al. (12) who find that a supernatant protein fraction partially purified from lysates of T2 or T4 phage-infected E. coli inhibits the response of normal E. coli core enzyme to σ.

During studies of new small proteins that bind to RNA polymerase after T4 phage

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infection (13), we have examined in detail polymerase from E. coli infected with a T4 Do (early) amber mutant. The results reported here describe briefly the σ-band content and activity of the polymerase at different purification stages. Assays of σ-containing and core enzyme fractions derived from both normal polymerase and polymerase from the T4-infected system show that the effect of normal σ on both core enzymes is modified by the σ-containing fraction of the enzyme from infected cells.

EXPERIMENTAL

Cells infected with T4 am N55-A456 (gene 42^- X gene 47^-) (the gift of Dr. John S. Wiberg) were obtained by growing E. coli B as previously described (13) at 30° in a 30-liter fermentor. When the cell concentration reached 3-4 X 10^8/ml, an 8-fold multiplicity of phage was added. The cells were collected 25 min after infection. Surviving cells were plated at 5 min following infection and were less than 1%. Uninfected E. coli B was grown in the same medium to a cell concentration of 7 X 10^8/ml.

The purification of the enzymes was essentially as described previously (13) and will be described in more detail elsewhere (Stevens, manuscript in preparation). Following a protamine sulfate precipitation step, the enzyme fractions were chromatographed on a DEAE-cellulose column, and the peak fractions were concentrated and further purified by glycerol gradient centrifugation. The gradient-purified enzymes contained no detectable DNase activity as analyzed by alkaline density-gradient centrifugation (Stevens, manuscript in preparation). The DEAE-cellulose and gradient-purified enzymes were found to contain negligible polynucleotide phosphorylase activity as determined by the polymerization assay of Williams and Grunberg-Manago (14). Phosphocellulose column chromatography was carried out as described by Burgess et al. (1), using a 0.7 X 5-cm column and applying approximately 1 mg of enzyme after glycerol gradient purification.

RESULTS AND DISCUSSION

Figure 1 (A and B) shows densitometer scans of SDS-polyacrylamide gels of RNA polymerase (T4-enzyme) prepared from T4 am N55-A456-infected cells at two stages of the purification procedure. The enzyme is shown after DEAE chromatography in Fig. 1A and after further purification by glycerol density-gradient centrifugation in Fig. 1B. The main polymerase subunits are as designated, and the new small binding proteins (13) are identified on the basis of their molecular weights of 22, 15, 12, and 10 (X 10^3), respectively, on the 12% gels. The T4-enzyme after the DEAE step (Fig. 1A) contains substantial amounts of a band migrating in the same position as normal σ. The σ-band content (about 0.4 equivalent/enzyme molecule) is close to that measured on enzyme isolated from normal

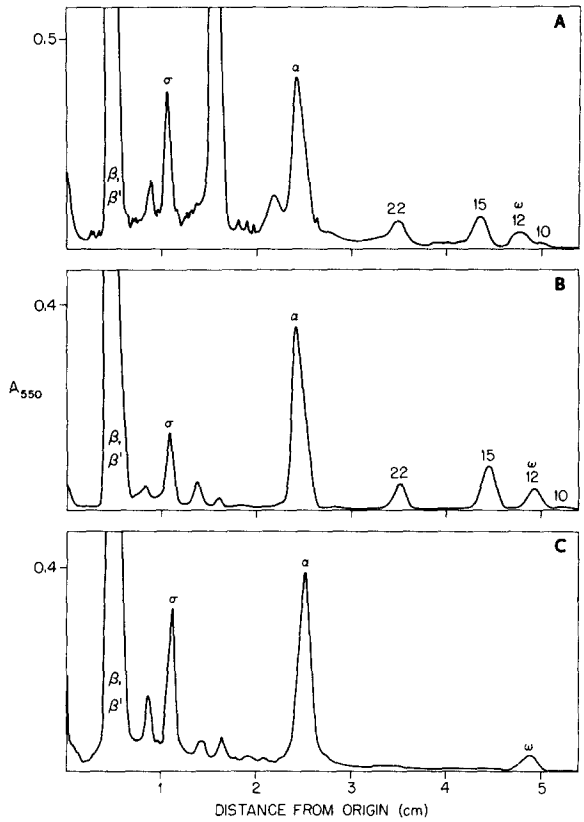

FIG. 1. A and B, Densitometer scans of stained gels of RNA polymerase isolated from cells infected with T4 phage am N55-A456 (gene 42⁻ X gene 47⁻). A, The enzyme (10 μg) was purified through the DEAE chromatography step. B, The enzyme (12 μg) was further purified by a glycerol gradient centrifugation step. (Numbers above peaks are mol wt X 10^3.) C, Densitometer scan of a stained gel of RNA polymerase isolated from normal E. coli B. The enzyme (15 μg) was purified through the glycerol gradient centrifugation step. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and staining and scanning of the gels was carried out as previously described (13).

E. coli (normal enzyme) after either the DEAE-cellulose step or the gradient-centrifugation step (Fig. 1C). (The large band in the middle of the gel in Fig. 1A is an unidentified impurity.) After gradient centrifugation of T4-enzyme (Fig. 1B), the σ content is reduced by about 2/3. In spite of having an apparently high σ-band content at early purification stages, the enzyme does not have the same activity with T4 DNA as template as does normal enzyme. The enzyme shown in Fig. 1A was assayed as described previously (13) and had a specific activity (nmoles of [^{14}C]AMP incorporated/mg protein/10 min at 37°) of approximately 90 with T4 DNA. Normal enzyme at the same purification stage had a specific activity of about 250. The enzyme shown in Fig. 1B also had a specific activity of 90 while normal enzyme (Fig. 1C) had a specific activity of 400. Using calf thymus DNA as template, the

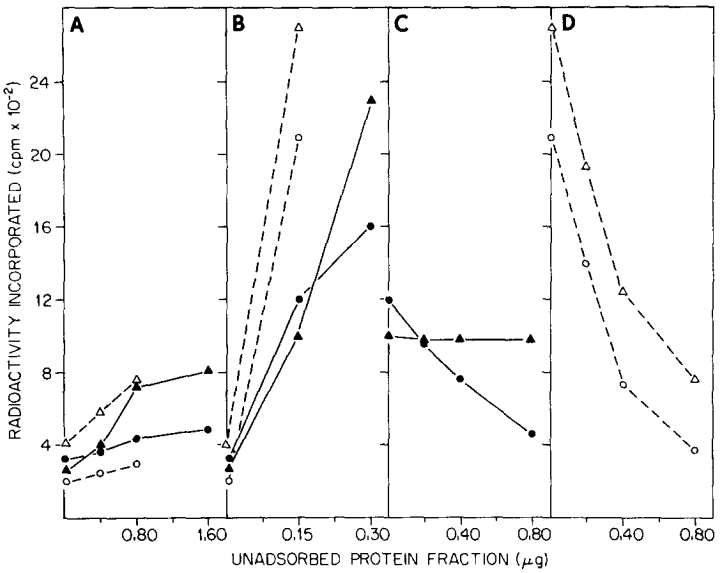

FIG. 2. A, Effect of the unadsorbed fraction of T4-enzyme on the activities of T4- and normal-core enzymes with T4 DNA as template. The reaction mixtures (0.2 ml) contained [^{14}C]ATP, 0.25 mM, 3400 cpm/nmole; UTP, CTP, GTP, each 0.25 mM; Tris buffer (pH 7.7) 20 mM; $MgCl_2$, 10 mM; β-mercaptoethanol, 10 mM; T4 DNA isolated from T4D phage, 8-10 μg; and KCl and enzyme as described. Incubation was for 10 min at 37°, and determination of the radioactivity incorporated into RNA was carried out as previously described (15). The σ content of the unadsorbed fraction was approximately 40% as determined by gel electrophoresis. ●——●, 0.03 M KCl, T4-core enzyme, 3 μg; ▲——▲, 0.03 M KCl, normal-core enzyme, 4 μg; O ---- O, 0.15 M KCl, T4-core enzyme, 3 μg; △----△, 0.15 M KCl, normal core enzyme, 4 μg. B, Effect of the unadsorbed fraction of normal enzyme on the activities of T4- and normal-core enzymes with T4 DNA as template. The reaction mixtures were as in (A) and the σ content of the unadsorbed fraction was about 70% as determined by gel electrophoresis. Designations are the same as in (A). C, Effect of the unadsorbed fraction of T4-enzyme on the normal σ-stimulated activity of the two core enzymes at low KCl concentration (0.03 M). The reaction mixtures were as in (A). Normal σ fraction (0.15 μg) was present in all the reactior mixtures. The σ content of the unadsorbed fraction of T4-enzyme and the amounts and designations of core enzymes are similar to A. D, Effect of the unadsorbed fraction of T4-enzyme on the normal σ-stimulated activity of the two core enzymes at high KCl concentration (0.15 M). The details of the assays are as described in (C).

enzymes shown in Fig. 1A, 1B, and 1C had specific activities of 250, 400, and 400 respectively. The results suggested that, if the protein migrating like σ is indeed σ, its activity is reduced on the T4-enzymes. More details of the measurements of σ-band content will be presented elsewhere (Stevens, manuscript in preparation).

Phosphocellulose column chromatography of normal enzyme and T4-enzyme, both purified by gradient centrifugation, was carried out to obtain σ-containing and core enzyme fractions. The major portion of the σ band is removed from both enzymes and appears in the

unadsorbed fraction while the core enzyme is eluted at high salt concentration. With T4-enzyme, the small protein of 12,000 mol wt and the residual amount of the one with 10,000 mol wt are also found in the unadsorbed fraction. Only very small amounts of the 22,000 mol wt protein are found in the unadsorbed fraction, while with T4-enzyme at earlier purification stages, most of this protein is in that fraction. The impurity bands shown in Fig. 1 (B and C) are found in the unadsorbed fraction.

The effects of the two unadsorbed (σ-containing) fractions on the activities of the two core enzyme fractions with T4 DNA as template are shown in Fig. 2(A and B). With a low concentration of KCL (0.03 M) in the reaction mixtures, the unadsorbed protein of T4-enzyme stimulates the two core enzymes poorly, about 3-fold at best (Fig. 2A). Normal σ fraction is very stimulatory to both core enzymes (Fig. 2B). In the presence of higher KCl (0.15 M), the activity of the T4-core enzyme is inhibited about 40% and the stimulatory activity of the corresponding unadsorbed fraction remains about the same. Normal core enzyme activity is stimulated about 50% by 0.15 M KCl, and the extent of stimulation by the unadsorbed protein from T4-enzyme is slightly reduced (Fig. 2A). Normal σ fraction stimulates both core enzymes better at 0.15 M KCl (Fig. 2B). The unadsorbed protein fractions had only negligible activity (10-30 cpm).

The effect of normal σ on both core enzymes was modified by the unadsorbed fraction from the T4-enzyme. At low KCl (0.03 M) (Fig. 2C), the fraction is inhibitory to the normal σ-stimulated activity of the T4-core enzyme. Using twice the amount of normal σ, the inhibition by the unadsorbed fraction was reduced about 30%. No inhibition of the activity of the normal core enzyme was found. At 0.15 M KCl, (Fig. 2D), the normal σ-stimulated activity of both core enzymes is inhibited by small amounts of the unadsorbed protein of T4-enzyme. Preincubation of core enzymes and normal σ reduces the inhibition more than 50%.

Unadsorbed protein with this inhibitory activity was also detected in the T4-enzyme at two earlier steps of purification, the protamine sulfate and DEAE cellulose steps. The inhibitory material sediments to the same position as σ in glycerol density gradients, but, at this time, there is no evidence for modification of σ. It is possible that σ may be closely associated with small amounts of one of the new small binding proteins or one of the unidentified impurity bands. Further purification of the fractions is in progress to characterize the nature of the inhibitor and its mode of action.

This inhibitory activity associated with highly-purified enzyme from T4 phage-infected cells may be similar to the anti-σ activity previously described in polymerase-free protein fractions from T2 or T4 phage-infected cells (8). Also, Mahadik et al. (16) have described

a partially purified protein fraction from T3-infected cells which inhibits E. coli RNA polymerase, possibly by antagonizing the action of α. It is unlikely that the modification would be unique to this particular T4 phage amber mutant infection system, but, at this time, we have studied only one other T4 phage-infected system in detail. Enzyme isolated from a T4 phage am BL 292 (gene 55⁻)-infected system and lacking the small binding protein of 22,000 mol wt (13) contains less of the inhibitory activity than the enzyme described here. A complete description of these studies will be reported (Stevens, manuscript in preparation).

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