Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase of Caulobacter crescentus

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Deoxyribonucleic acid-dependent ribonucleic acid (RNA) polymerase (EC 2.7.7.6) was purified from the dimorphic bacterium Caulobacter crescentus at three stages in development. Enzyme from pure populations of stalked cells, as well as populations enriched in swimmer and predivisional cells, appeared identical in subunit structure and template requirements. The molecular weights of the enzyme subunits were 165,000, 155,000, 101,000, and 44,000, respectively. By analogy with RNA polymerase from other bacterial sources, they are considered to be components of the C. crescentus holoenzyme, β', β, α, and α, respectively. The C. crescentus enzyme appeared similar to the Pseudomonas aeruginosa enzyme and unlike the Escherichia coli enzyme with respect to subunit molecular weights and failure to separate into core and sigma components upon phosphocellulose chromatography. In addition, the effects of ionic strength on the time course of polymerization varied both with the sources of bacterial polymerase and bacteriophage DNA.

The life cycle of the bacterium Caulobacter crescentus involves at least three differentiated cell types (Fig. 1). The flagellated swimmer cell matures into a stalked cell, with a concomitant loss of motility. The stalked cell then develops into a predivisional cell by the synthesis of pili and flagella at the pole opposite the stalk. The predivisional cell then divides to yield one stalked and one swimmer cell. The three cell types differ with regard to the presence of membranous organelles (16, 17), presence of cell wall receptor sites for the deoxyribonucleic acid (DNA) phage φCbK (1, 20), rate of DNA synthesis (5), and sodium dodecyl sulfate (SDS) gel patterns of whole cellular protein (21; unpublished observations). Since new ribonucleic acid (RNA) synthesis appears to be required for the expression of differentiation events (15), it was of interest to determine whether a bacterium which must undergo these morphological and biochemical changes utilizes multiple RNA polymerases for selective transcription.

This report describes the purification and characterization of RNA polymerase from C. crescentus stalked cells, as well as cell cultures composed mainly of either swimmer or predivisional cells. These enzyme preparations have been compared to each other and to the Escherichia coli RNA polymerase with regard to behavior on phosphocellulose, subunit structure, template specificity, and the effects of ionic strength on the reaction kinetics.

MATERIALS AND METHODS

Materials. Nonradioactive ribonucleoside triphosphates, as well as the 32P-labeled adenosine 5'-triphosphate (ATP), guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), and uridine 5'-triphosphate (UTP), were obtained from Schwarz-Mann. Phosphoenolpyruvate, dithiothreitol, and rifampin (B grade) were obtained from Calbiochem; protamine sulfate was from Eli Lilly. Whatman diethylaminoethyl (DEAE)-cellulose DE-23 and DE-52 and phosphocellulose P-11 were obtained from Reeves-Angel. Bovine serum albumin was obtained from Armour Pharmaceutical as a sterile solution at 300 mg/ml. Phenol was obtained from Fischer Scientific and redistilled before use. Acrylamide and bisacrylamide were from Eastman Organic Chemicals and Coomassie brilliant blue was from Colab Laboratories. Ribonuclease (RNase)-free sucrose was obtained from Schwarz. Whatman GF/C filters were from Reeves-Angel. Omnifluor was obtained from New England Nuclear, and tolidine (scintanalyzed grade) was from Fischer Scientific.

Dialysis tubing obtained from Arthur H. Thomas was subjected to three cycles of boiling in Na2CO3 + 0.01 M ethylenediaminetetraacetic acid (EDTA) for 15 min and washing in deionized water: it was then autoclaved in 1 mM EDTA for 20 min and stored in 1 mM EDTA until use. Alumina, purchased from Alcoa,
was washed in four changes of 0.01 M HCl over several hours and then with deionized water until a neutral pH was attained. After collection on a Buchner funnel, the alumina was dried in an oven at 60°C and stored at -20°C until use. Acid-washed glass beads and AmSO₄ (enzyme grade) were obtained from Schwarz-Mann.

Bovine pancreatic RNase, pancreatic deoxyribonuclease (DNase) I, and pyruvate kinase were obtained from the Worthington Biochemical Corp. E. coli RNA polymerase was the generous gift of L. Yarborough; SP15 and SP82 DNAs were kindly provided by J. Marmur. Poly d(AT) was purchased from Miles Laboratories. Q8-H-RNA was the gift of G. Kuo.

**Bacterial strains and growth conditions.** C. crescentus strain CB13Blα was grown on PYE complex medium (17) at 30°C with aeration. Pure populations of stalked cells were prepared from stationary phase cultures (17, 19). Cells were harvested by centrifugation and frozen at -4°C. Prediisional cells were prepared from nonmotile stationary phase cultures which had been diluted 1:2 with fresh PYE broth, prewarmed to 30°C, and allowed to grow. When motility was first observed in the phase-contrast microscope, approximately 40 min after the optical density began to rise and shortly after the onset of flagellin synthesis (21), the cells were harvested as above. This method yields approximately 80% prediisional cells. This synchronization technique was also employed to obtain gram quantities of partially purified swarmer cells. The diluted cultures were allowed to grow for approximately 80 min after the optical density began to rise and were then separated from the stalked cells by differential centrifugation (17, 19). Swarmer cells obtained by this method were approximately 70% pure.

**Preparation of bacteriophage DNA.** Coliphage T2 and Caulobacter phage φCbK were grown on E. coli B and CB13Blα, respectively, under standard conditions, and φCbK was purified as previously described (1). T2 phage were purified by differential centrifugation and resuspended in a buffer [10 mM tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.4, 0.3 M KCl, and 5 mM MgCl₂] to an absorbance of approximately 15.0. This was followed by equilibrium sedimentation in CsCl at 40,000 rpm for 48 h. After density flotation, T2 or φCbK phage was dialyzed against 50 mM NaCl, 10 mM Tris buffer, pH 8, and 0.1 mM EDTA (buffer D). The phage DNA was extracted twice with redistilled phenol and then equilibrated with buffer D. The DNA was extensively dialyzed against buffer D and stored over chloroform at 4°C.

**Assay of RNA polymerase.** The standard reaction mixture (0.1 ml) contained 40 mM Tris-hydrochloride buffer (pH 8), 10 mM MgCl₂, 10 mM β-mercaptoethanol, 0.5 mM each of ATP, GTP, CTP, and UTP, and 15 to 20 nmol of DNA. In each experiment one of the nucleoside triphosphates was ³²P-labeled at a specific activity of 2,400 to 4,400 counts per min per nmol. When highly purified enzyme preparations were assayed, 6 μg of bovine serum albumin was added to the assay mixture to stabilize the enzyme. After incubation at 37°C for 10 min the reaction was stopped by the addition of 2.5 ml of cold 3.5% perchloric acid containing 0.1 M inorganic pyrophosphate. The samples were mixed vigorously after at least 10 min at 0°C, and acid-insoluble material was collected in GF/C glass fiber filters which had been soaked with 1 M HCl containing 0.1 M inorganic pyrophosphate (HCl + PP₃). The tube was rinsed four times with a total of 25 ml of HCl + PP₃, with mixing, and the filter was then washed three times with 5-ml samples of HCl-PP₃, and once with ethanol. Filters were dried and counted in 6 ml of a solution containing 4 g of Omnifluor per liter of toluene. One unit of RNA polymerase activity is defined as that amount of enzyme required to catalyze the incorporation of 1 nmol of guanosine 5'-monophosphate (GMP) into an acid-insoluble product in 10 min at 37°C. The specific activity is the number of units per milligram of protein; protein was measured by the method of Lowry et al. (13) with bovine serum albumin as the standard.

**Polyacrylamide gel electrophoresis.** A modification of the procedure of Shapiro et al. (18) was used. Five percent polyacrylamide gels, 0.1% in SDS and 0.1 M in sodium phosphate buffer, pH 7.1, were prepared in tubes 6 mm in diameter and stored at 4°C over water until needed. Samples were then brought to a final concentration of 4% sucrose, 0.01% bromophenol blue, 0.02 M phosphate buffer (pH 7.1), 0.1% SDS, and 0.01 M β-mercaptoethanol in a volume of 0.1 ml and incubated at 100°C for 2 min. Where necessary, samples were precipitated with 10 to 15% trichloroacetic acid and centrifuged. The pellets were resuspended in the incubation buffer described above and brought to neutral pH by the addition of 5 to 10 uliters of 1 M sodium phosphate buffer. They were
then incubated as described above. Each sample contained 10 to 20 μg of protein. Electrophoresis was carried out in 0.1 M sodium phosphate buffer (pH 7.1), 0.05% SDS, and 0.01 M β-mercaptoethanol at 8 to 8.5 mA/gel (50-60 V) by using a Heathkit model 1P-17 power source. Alternatively, 3-mm diameter gels were prepared and run at 4 mA/gel (50-60 V); in this case each gel contained 2 to 5 μg of protein.

After electrophoresis for approximately 5.5 h gels were allowed to equilibrate with a solution containing methanol-acetic acid-water (200 ml: 40 ml: 200 ml: vol/vol/vol), followed by staining for 3 h in the same solution containing 1.1 g of Coomassie brilliant blue dye. They were electrophoretically destained for 40 min in a solution containing 7.5% acetic acid and 5% methanol, with a buffer change at 20 min using a Cana1co apparatus with the model 20 power source. Gels were stored in destaining solution.

RESULTS

Purification of C. crescentus RNA polymerase. RNA polymerase was purified by a modification of the method of Berg et al. (2). Buffer A contained 10% glycerol, 0.05 M Tris-hydrochloride (pH 8), 0.01 M MgCl₂, 10⁻⁴ M dithiothreitol, and 10⁻⁴ M EDTA. Buffer B contained 10% glycerol, 0.02 M Tris (pH 8), 0.01 M MgCl₂, 10⁻⁴ M dithiothreitol, and 10⁻⁴ M EDTA. Storage buffer contained buffer B with 60% (vol/vol) glycerol. Buffer C contained 5% glycerol, 0.05 M Tris (pH 8), 10⁻⁴ M dithiothreitol, and 10⁻⁴ M EDTA. Saturated ammonium sulfate solution contained 4.1 M (NH₄)₂SO₄ over solid (NH₄)₂SO₄ at 25 °C plus sufficient NaOH to bring the pH to 7.5. All manipulations were carried out at 0 to 4 °C. Centrifugations were for 20 min at 15,000 rpm in a Sorvall SS-34 rotor unless otherwise specified.

(i) Crude extract. Frozen C. crescentus stalked cells (55.5 g) were ground with 107 g of alumina, and the resulting paste was suspended in 150 ml of buffer A. After centrifugation for 60 min, the supernatant fluid was saved and the pellet was washed with 60 ml of buffer A and centrifuged once again for 60 min. The supernatant fluids were combined (fraction I, 200 ml). When enzyme was isolated from 100 g or more of cells, the cells were processed with a Waring blender and glass beads (2) with similar results. Extracts of swarmer or preswimming cells were prepared in the same manner with 5 g or 10 g of cells, respectively.

(ii) DEAE-cellulose extraction. To fraction I (200 ml), 28.6 ml of 2 M KCl was added, with mixing, followed by 116 g of moist DE-52 DEAE, pre-equilibrated with buffer A + 0.26 M KCl. The slurry was stirred gently for 20 min and filtered through a Buchner funnel with pressure applied from above. The DEAE was washed twice with a total of 120 ml of buffer A. As each 50-ml portion was eluted, 7 g of ammonium sulfate and 0.07 ml of 1 N NaOH were added, and the resultant suspensions were centrifuged. To the pooled supernatant fractions (335 ml) were added 81 g of ammonium sulfate and 0.81 ml of 1 N NaOH. The resultant suspension was centrifuged for 120 min. The pellets were washed twice with approximately 50 ml of buffer A + 1.3 M (NH₄)₂SO₄ per wash. The resultant 110 ml of supernatant fluid was brought to 2.0 M by adding 12 g of (NH₄)₂SO₄ and 0.12 ml of 1 N NaOH and centrifuging for 2 h. The pellets were thoroughly drained of supernatant solution and suspended in buffer B (fraction II, 385 ml).

(iii) Proteamine precipitation. Microtitrations of proteamine precipitation carried out on 0.25-ml samples of fraction II indicated that 16 ml of proteamine sulfate (1.0%) should be added to 385 ml of fraction II. The resulting suspension was stirred for 30 min and centrifuged. The precipitate was washed for 45 min with 35 ml of buffer A + 0.1 M magnesium acetate and centrifuged. Two elutions of the resulting pellet with buffer A + 0.1 M ammonium sulfate, followed by centrifugation, were performed using 33 ml for the first and 17 ml for the second elution. The combined supernatant fractions comprised fraction III (52 ml).

(iv) Ammonium sulfate fractionation. A saturated ammonium sulfate solution (61 ml) was added to fraction III and stirred for 15 min, and the resulting suspension was centrifuged. The pellet was washed successively with 22.5 ml of buffer A + 2.0 M (NH₄)₂SO₄, 15 ml of buffer A + 1.8 M (NH₄)₂SO₄, 15 ml of buffer A + 1.6 M (NH₄)₂SO₄, 10 ml of buffer A + 1.6 M (NH₄)₂SO₄, 8 ml, then 8 ml, and then 9 ml of buffer A + 1.5 M (NH₄)₂SO₄, and finally 8 ml of buffer A + 1.4 M (NH₄)₂SO₄. The 1.6 M and 1.5 M eluates, which contained 85% of the recovered polymerase activity, were pooled, brought to 60% saturation in ammonium sulfate and centrifuged. The precipitate was suspended in buffer B to a final volume of 10 ml (fraction IV).

(v) DEAE-cellulose chromatography. Fraction IV was diluted to 50 ml and loaded onto a DEAE-cellulose column (1 x 13 cm) previously equilibrated with buffer B. The column was washed successively with 7.5 ml of buffer B, 25 ml of buffer B + 0.05 M KCl, and a 250-ml linear gradient of 0.05 M to 0.4 M KCl in buffer B at a flow rate of approximately 10 ml/h. Enzyme activity was eluted at approximately 0.25 M KCl, and the fractions containing maximum specific activity (10 ml) were pooled. The enzyme was precipitated by adding 1.2 vol of saturated (NH₄)₂SO₄ and dissolved in storage buffer at a protein concentration of about 5
ml/ml (fraction V'). The SDS-polyacrylamide gel pattern of enzyme purified through the DEAE step is shown in Fig. 2. DEAE enzyme was stable for at least 3 months at -20°C.

In the preparation of enzyme from swarmer (4 g) and predivisional cells (10 g) for which the material was limited, the DEAE chromatography was performed with stepwise elution. A summary of the purification of the stalked cell enzyme preparation appears in Table 1.

(vi) Phosphocellulose chromatography. To the peak fractions of the DEAE-cellulose eluate, fraction V', which had a specific activity of 950 (7.5 mg of protein), was added 1.3 vol of saturated ammonium sulfate solution. After 15 min at 0°C the suspension was centrifuged. The pellet was resuspended in 0.5 ml of buffer C and rapidly dialyzed against four changes of 100 ml each of buffer C. Approximately 20% of the enzyme activity was inactivated by the dialysis. Sufficient buffer C was added to decrease the conductivity to that of buffer C + 0.05 M KCl. The enzyme (fraction V') (2.4 ml) was then applied at a rate of 0.04 ml/min to a phosphocellulose column (1 by 10 cm) previously equilibrated with buffer C + 0.05 M KCl. The enzyme was then allowed to equilibrate with the column for 30 min. The column was washed successively with buffer C containing 0.05 M KCl, 0.15 M KCl, 0.27 M KCl, and 0.35 M KCl, at a flow rate of 0.07 ml/min. Buffers were changed when the optical density at 280 nm of the eluate equalled that of the applied buffer; at least 25 ml of each buffer was applied. Fractions containing maximum specific activity were pooled, precipitated with 1.3 vol of saturated (NH₄)₂SO₄, and resuspended in storage buffer to a protein concentration of 0.6 to 1.0 mg/ml. No protein or enzyme activity was eluted with 0.15 M KCl, and total enzyme units were recovered in the 0.27 M KCl and 0.35 M KCl eluates. The flow-through fractions failed to stimulate either the 0.27 M or 0.35 M KCl eluate fractions, indicating that the C. crescentus polymerase was not separated into core and

![Fig. 2. SDS-polyacrylamide gel electrophoresis of RNA polymerase from C. crescentus stalked cells. The gels contained 15 μg of fraction V (DEAE) enzyme (A); 10 μg of 0.05 M KCl phosphocellulose eluate (B); 10 μg of fraction VI-A (phosphocellulose 0.27 M KCl eluate) enzyme (C); 4 μg of fraction VI-B (phosphocellulose 0.35 M KCl eluate) enzyme (D). The gels (6 mm) were run and stained by the procedure described in Materials and Methods.](http://jb.asm.org/)

### Table 1. Purification of RNA polymerase from C. crescentus

<table>
<thead>
<tr>
<th>Enzyme fraction</th>
<th>Units (nmol/10 min)</th>
<th>Specific activity (units/mg of protein)</th>
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</thead>
<tbody>
<tr>
<td>I Crude extract</td>
<td>39,000</td>
<td>7.7</td>
</tr>
<tr>
<td>II DEAE eluate</td>
<td>20,800</td>
<td>18.3</td>
</tr>
<tr>
<td>III Protamine sulfate eluate</td>
<td>20,800</td>
<td>200.0</td>
</tr>
<tr>
<td>IV (NH₄)₂SO₄-back fraction</td>
<td>13,700</td>
<td>333.0</td>
</tr>
<tr>
<td>V DEAE eluate</td>
<td>17,200</td>
<td>525.0</td>
</tr>
<tr>
<td>V' Peak fractions of DEAE eluate (dialyzed)</td>
<td>5,583</td>
<td>745.0</td>
</tr>
<tr>
<td>VI Phosphocellulose</td>
<td>0.27 M eluate</td>
<td>4,480</td>
</tr>
<tr>
<td></td>
<td>0.35 M eluate</td>
<td>1,045</td>
</tr>
</tbody>
</table>
In addition, T2 was isolated by phosphocellulose chromatography. This conclusion was supported by the fact that the ratio activity of poly d(AT) versus T2 was the same for the applied enzyme, the 0.27 M KCl eluate, and the 0.35 M KCl eluate. In addition, the gel patterns of these fractions (Fig. 2) show that proteins analogous to α, β, β', and σ subunits are all present in both the 0.27 M and 0.35 M KCl eluates, and none of them are present in the flow-through.

The phosphocellulose step for enzyme purified from predisional cells was similar to that described above, except that no 0.15 M KCl wash was performed. Also, because of the small quantity of enzyme available from this source, a 4-cm-long column in a Pasteur pipette was used with a loading flow rate of 0.015 ml/min and a washing flow rate of 0.31 ml/min. At least 2.5 ml of each washing buffer was used. Here again all activity was recovered in the 0.27 M KCl and 0.35 M KCl eluates, and the enzyme contained α, β, β', and σ subunits (Fig. 3).

**Properties of the reaction.** Purified RNA polymerase of *C. crescentus* stalked cells was totally dependent on added DNA as template. With d(AT) copolymer as template, the molar incorporation of 14C-uridine 5'-monophosphate was exactly equal to that of 14C-adenosine 5'-monophosphate. Reaction mixtures incubated with deoxyribonuclease prior to addition of enzyme failed to catalyze nucleotide incorporation. Incubation of the reaction product with ribonuclease rendered it totally acid soluble. In addition to template DNA, all four triphosphates and Mg2+ were required for GMP incorporation. Maximal incorporation was observed at MgCl2 concentrations of 10 to 70 mM, inclusive, and MnCl2 at concentrations of 0.2 to 40 mM was not an effective substitute for MgCl2. Incorporation catalyzed by *C. crescentus* DNA-dependent RNA polymerase was sensitive to the drug rifampin (10 μg/ml). Polynucleotide phosphorylase or polyadenylate polymerase activities were not detected. The $K_m$ of the *C. crescentus* RNA polymerase phosphocellulose fraction for CTP was found to be $2.4 \times 10^{-5}$ M.

**Subunits of RNA polymerase.** A comparison of the SDS gel patterns of the *E. coli* and *C. crescentus* RNA polymerases shows molecular weight differences in the α and σ subunits (Fig. 4). From a standard plot of distance migrated versus log molecular weight, the molecular weights of the *C. crescentus* β', β, σ, and α subunits were estimated to be 165,000, 155,000, 101,000, and 44,000, respectively. Similar values have been obtained for the subunits of *Pseudomonas putida* polymerase, namely, 165,000, 155,000, 98,000, and 44,000 (7). Molecular weights of 165,000, 155,000, 95,000 and 39,000, respectively, were assumed for the *E. coli* enzyme in both studies.

The subunit structure of *C. crescentus* RNA polymerase was shown to be 165,000, 155,000, 155,000, 95,000 and 39,000, respectively. These values are in agreement with the molecular weights of the subunits of *Pseudomonas putida* polymerase. The subunit structure of *C. crescentus* RNA polymerase was also determined by SDS-polyacrylamide gel electrophoresis. The gels (3 mm) contained 4 μg of *C. crescentus* fraction VI-A (phosphocellulose 0.27 M KCl eluate) enzyme purified from stalked cells (A); 2 μg of *C. crescentus* fraction VI-A enzyme purified from predisional cells (B); 4 μg of fraction VI-A stalked cell enzyme plus 2 μg of fraction VI-A predisional cell enzyme (C). The gels were run and stained as above (Fig. 2).
polymerase was studied by SDS-polyacrylamide gel electrophoresis of the purified enzyme obtained from three distinct cell types: stalked cells, predivisional cells, and swarmer cells. Parallel gels containing enzyme from stalked cells and predivisional cells were run both separately and together (Fig. 3). Using this criterion, there appear to be no differences in subunit molecular weight between the enzymes isolated from these two sources. A similar result was obtained when DEAE eluate fractions from swarmer and stalked cells were compared (Fig. 5). The contaminants, including the major contaminating band migrating between the \( \sigma \) and \( \beta \) positions, were also observed in stalked and predivisional DEAE enzyme preparations and, in these cases, were removed by phosphocellulose chromatography. The limited quantities of swarmer cells available prevented further purification of swarmer-derived polymerase past the DEAE step. The culture of swarmer cells from which the swarmer RNA polymerase was obtained was approximately 70% pure. Under these conditions an altered subunit would represent only a portion of the total bands observed. However, the major band distribution on swarmer and stalked cells are identical. It would thus appear that if any changes in RNA polymerase do occur during Caulobacter differentiation, they do not involve major alterations in subunit structure, such as has been reported for B. subtilis (9, 12).

**Template specificity.** RNA polymerase purified from C. crescentus stalked, predivisional, and swarmer cells, and from E. coli, had similar activity with template DNA of widely differing sources. Native \( \phi \)CbK DNA was an efficient primer for all the enzyme preparations, and was approximately one-fourth as active in the denatured as in the native form (Table 2). The enzymes were approximately equally active on DNA isolated from coliphage T2. The synthetic copolymer, poly d(AT), was an efficient primer, although somewhat less so for the C. crescentus stalked cell enzyme than for the other enzyme preparations. The DNA of B. subtilis phage SP82 was an efficient template, but B. subtilis phage SP15 DNA was a very poor template for all of the enzymes studied; similar results have been obtained with the RNA polymerases of Azotobacter vinlandii (J. Krakow, personal communication) and Bacillus subtilis (J. Pène, personal communication). SP15 DNA lacks thymine, having instead a 5-dihydroxypentose-uracil derivative with a disaccharide phosphate attached to the pentose moiety, probably via a phosphodiester linkage (C. Brandon, personal communication).

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**Fig. 4.** SDS-polyacrylamide gel electrophoresis of RNA polymerases of C. crescentus and E. coli. The gels (3 mm) contained 2.2 \( \mu \)g of C. crescentus fraction VI-A (phosphocellulose 0.27 M KCl eluate) enzyme (A); 2.8 \( \mu \)g of E. coli enzyme (B); 2.0 \( \mu \)g of C. crescentus enzyme plus 1.2 \( \mu \)g of E. coli enzyme (C); 2.0 \( \mu \)g each of C. crescentus and E. coli enzymes (D); 2.0 \( \mu \)g of C. crescentus enzyme plus 2.8 \( \mu \)g of E. coli enzyme (E). The gels were run and stained as above (Fig. 2).
The template requirements of *C. crescentus* RNA polymerase did not change upon phosphocellulose chromatography (Table 2). A comparison of enzyme activity with poly d(AT) or T2 DNA as template revealed comparable d(AT)/T2 ratios for DEAE (fraction V) enzyme and the two phosphocellulose fractions VI-A and VI-B. This is in keeping with the proposed holoenzyme structure of the enzyme in these fractions. In contrast to these findings with the *C. crescentus* enzyme, it has been previously shown that the *E. coli* polymerase holoenzyme is resolved into σ and core components by phosphocellulose chromatography. The core enzyme, which is eluted from phosphocellulose at approximately 0.33 to 0.35 M KCl, has a characteristically high poly d(AT)/T2 ratio (2-4).

The stalked cell enzyme was somewhat more active on φCbK DNA and less active on poly d(AT) than were the other enzyme preparations. To test the possibility that this observation was due to a contaminating activity (e.g., nuclease) in the stalked cell enzyme preparation, the following experiments were performed. The DNA template to be tested was incubated with *E. coli* polymerase, *C. crescentus* stalked cell (Fraction VI-A) polymerase, or a mixture of both enzyme preparations. Whether the template was poly d(AT), φCbK, or T2 DNA, the nucleotide incorporation obtained when the enzymes were incubated together was equal to the sum of the nucleotide incorporations when the enzymes were assayed separately. Additive results were also obtained in similar assays using *C. crescentus* stalked cell fraction V and predivisional cell fraction IV, with φCbK and T2 DNA as the templates.

**Effect of salt on time course of reaction with coliphage and Caulobacter phage DNA templates.** Whereas no significant differences in the template specificities of the *E. coli* and *C. crescentus* enzymes were detected in standard assay conditions, major differences were detected after longer incubation times or at different ionic strengths. Thus, 0.15 M KCl reduced the yield of coliphage T2 DNA-directed RNA product in the reaction catalyzed by the *C. crescentus* polymerase, but enhanced the yield in that catalyzed by the *E. coli* enzyme (Fig. 6). Sucrose gradient analysis showed that, with the *E. coli* enzyme, extensive RNA chain release occurred at 0.15 M KCl, whereas a much smaller, but significant, proportion of RNA chains was released when the reaction was incubated in the absence of salt. The salt-mediated stimulation of RNA product release is similar to results reported by Maitra et al. (14),

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**Fig. 5. SDS-polyacrylamide gel electrophoresis of RNA polymerase from *C. crescentus* stalked and swarmer cells.** The gels (6 mm) contained 15 μg of *C. crescentus* fraction V (peak DEAE) enzyme purified from stalked cells (A); 16 μg of fraction V (DEAE) enzyme purified from swarmer cells (B); 9 μg of fraction V stalked cell enzyme plus 16 μg of fraction V swarmer cell enzyme (C). The gels were run and stained as above (Fig. 2).
in which T4 DNA was used as template. In the presence of the C. crescentus polymerase, on the other hand, extensive release of T2-directed RNA occurred in both the absence and presence of salt.

With φCbK DNA as template the initial rate of synthesis and yield of the reaction catalyzed by the E. coli RNA polymerase were inhibited by high ionic strength (Fig. 7A). In contrast, RNA synthesis catalyzed by the C. crescentus RNA polymerase with φCbK DNA as template was generally unaffected by the ionic strength in that synthesis proceeded at a rapid rate for 30 to 40 min and then came to an abrupt halt, regardless of the salt concentration (Fig. 7B). The presence of 0.20 M KCl slightly inhibited both the initial rate of RNA synthesis and the final yield of RNA product. With either enzyme little or no RNA chain release occurred at any ionic strength. Treatment of the reaction mixtures with sodium dodecyl sulfate and mercaptoethanol for 5 min at 50 C released 80% of the product. The sedimentation profiles of φCbK-directed product RNA synthesized by the E. coli and C. crescentus polymerases are nearly identical, and both enzymes appear to synthesize longer RNA molecules at 0.15 M KCl than in the absence of salt. The addition of T2 or φCbK DNA to the reaction catalyzed by the C. crescentus enzyme did not promote further synthesis, indicating that a shortage of DNA was not responsible for the cessation of synthesis at 40 min (Table 3). The addition of more polymerase, however, resulted in the resumption of RNA synthesis and the amount of incorporation catalyzed in 40 min was the same.

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**Table 2. Template requirements of RNA polymerase purified from various sources**

<table>
<thead>
<tr>
<th>Source of RNA polymerase</th>
<th>φCbK*</th>
<th>T2*</th>
<th>poly d(AT)*</th>
<th>d(AT) ( \times 1.2^{n.8} )</th>
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<tbody>
<tr>
<td>A. DEAE eluate</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C. crescentus stalked</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction V, 1.6 µg</td>
<td>1.43</td>
<td>0.28</td>
<td>0.48</td>
<td>0.49</td>
</tr>
<tr>
<td>C. crescentus predivision</td>
<td>1.33</td>
<td>0.35</td>
<td>0.55</td>
<td>1.22</td>
</tr>
<tr>
<td>C. crescentus swarmer</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fraction V, 0.4 µg</td>
<td>0.28 (1.12)</td>
<td>0.09 (0.34)</td>
<td>0.25 (1.00)</td>
<td>2.8</td>
</tr>
<tr>
<td>E. coli, 1.8 µg</td>
<td>1.11</td>
<td>0.36</td>
<td>0.59</td>
<td>1.10</td>
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<tr>
<td>B. Phosphocellulose eluate</td>
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<td></td>
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<tr>
<td>C. crescentus predivision</td>
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<tr>
<td>Fraction VI-A, 0.8 µg</td>
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<tr>
<td>Fraction VI-B, 0.6 µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. crescentus stalked</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction VI-A, 1.0 µg</td>
<td>1.34</td>
<td>0.91</td>
<td>1.39</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Measured in nanomoles of GMP incorporated per 10 min in the case of φCbK and T2 DNA, and nanomoles of AMP incorporated per 10 min with poly d(AT).

The poly d(AT) and T2 preparations used in section B were, respectively, 2.2 and 1.8 times as active as those used in section A. To facilitate the comparison of the two experiments, the raw dAT/T2 ratios in section A were multiplied by 2.2/1.8 (≈1.2).
per microgram of enzyme as had occurred in the first 40 min of the reaction. It thus appears that the DNA template was not only present in sufficient quantity, but had not been damaged with respect to its priming ability during the 80 min preincubation period with enzyme. The possibility that *C. crescentus* RNA polymerase was inactivated by 40 min of incubation with φCbK DNA is not ruled out by these experiments, but is considered unlikely for the following reasons. (i) Enzyme inactivation would be expected to be a gradual process and not result in the abrupt shut-off of synthesis observed here. (ii) *C. crescentus* RNA polymerase was stable over at least 4 h of incubation at 37°C with T2 DNA; a loss of activity in the presence of φCbK DNA would have to be specific for that template. That the φCbK DNA preparations used did not contain agents which inactivate RNA polymerases in general is indicated by the prolonged incorporation obtained with the *E. coli* enzyme using this template.

**DISCUSSION**

DNA-dependent RNA polymerase isolated from *C. crescentus* is similar to other bacterial RNA polymerase enzymes in that four subunits were detected by SDS-polyacrylamide gel electrophoresis. By analogy with the known subunits of the *E. coli* enzymes, as well as polymerase from *P. putida* (7), *A. vinlandii* (8), and *B. subtilis* (12), the four subunits are assumed to correspond to β′, β, σ, and α, respectively. Based upon migration distance on SDS-polyacrylamide gels, the molecular weights of the σ and α subunits of the *C. crescentus* polymerase were 101,000 and 44,000, respectively; those of the *P. putida* polymerase were 98,000 and 44,000 (7), while the *E. coli* σ and α subunits were 95,000 and 39,000 daltons. RNA polymerase holoenzyme purified from *C. crescentus* swarmer, predivisional, and stalked cells behaved similarly to the *E. coli* RNA polymerase during the purification procedure, except that phosphocellulose chromatography did not separate the *C. crescentus* enzyme into sigma and core components. Maintenance of the holoenzyme was demonstrated by the following criteria. (i) There was complete recovery of enzyme activity measured on native templates after phosphocellulose chromatography. (ii) The ratio of enzyme activity using poly d(AT) versus T2 DNA as template was the same for the phosphocellulose eluates as for the unchromatographed enzyme. (iii) SDS-polyacrylamide gel electrophoresis revealed no sigma or other polymerase protein in the flow-through fractions. (iv) Enzyme fractions eluting at 0.25 to 0.27 M KCl and 0.35 M KCl all showed β′, β, σ, and α bands on gel electrophoresis. (v) No stimulatory activity could be found in the flow-through fractions. It has been demonstrated that phosphocellulose chromatography of holoenzyme isolated from *P. putida*, a close relative of *C. crescentus*, results in only a partial loss of the sigma subunit (7), whereas the holoenzyme of *A. vinlandii* (8), *B. subtilis* (12), and *E. coli* (2, 4, 11) are readily separated into

**TABLE 3. Effect of additional DNA or enzyme on RNA yield**

<table>
<thead>
<tr>
<th>Incubation* (min)</th>
<th>Addition at 80 min</th>
<th>GMP incorporation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No KCl</td>
<td>0.15 M KCl</td>
</tr>
<tr>
<td>20</td>
<td>0.74</td>
<td>0.76</td>
</tr>
<tr>
<td>40, 80, 120</td>
<td>1.23–1.25</td>
<td>1.24–1.26</td>
</tr>
<tr>
<td>120</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1.24</td>
<td>1.25</td>
</tr>
<tr>
<td>120</td>
<td>3.16</td>
<td>3.11</td>
</tr>
</tbody>
</table>

* A standard 0.1-ml reaction mixture containing 0.4 μg of *C. crescentus* stalked cell fraction VI-A RNA polymerase was incubated for the indicated period of time at 37°C. After 80 min of incubation, 10 μl of containing 10 nmol of φCbK, 10 nmol of T2 DNA, or 5.7 μg of *C. crescentus* stalked cell fraction VI-A enzyme was added and incubation was continued for another 40 min.

* Measured in nanomoles incorporated per 10 min.
sigma and core components on this resin. Experiments are in progress to separate the \textit{C. crescentus} polymerase into sigma and core by other means.

In addition to differing from the \textit{E. coli} RNA polymerase in (i) size of \(\sigma\) and \(\alpha\) subunits and (ii) dissociation of \(\sigma\) subunit by phosphocellulose chromatography, the \textit{C. crescentus} polymerase differs from the \textit{E. coli} enzyme with regard to the effect of salt on reaction rate and yield in the presence of two different bacteriophage DNA templates.

Studies of RNA polymerase from various \textit{Caulobacter} cell forms have shown that morphological and biochemical changes associated with development cannot be accounted for by major changes in DNA-dependent RNA polymerase. Differences in subunit composition or molecular weight were not detected among \textit{C. crescentus} swarmer, predivisional, and stalked cell polymerase preparations. Moreover, when holoenzyme preparations obtained from various \textit{C. crescentus} cell types were compared with respect to template specificity in a standard reaction, only minor differences in template preference could be detected. If changes in the structure of the \textit{C. crescentus} polymerase do in fact occur, they were not apparent in the cell types studied here or were not detected by the methods used to analyze the enzyme. This stands in contrast to the observed alteration of the \textit{B. subtilis} RNA polymerase upon the switch from vegetative growth to spore formation (6, 9, 10, 12). Alternatively, the control of gene expression apparent during morphogenesis may be attributable to other regulatory molecules, such as cyclic nucleotides and cyclic nucleotide binding proteins that have been shown to be involved in gene transcription in other organisms. These are currently being studied in relation to selective transcription during \textit{Caulobacter} development.

ACKNOWLEDGMENTS

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LITERATURE CITED


