Purification of the Gene 0.3 Protein of Bacteriophage T7, an Inhibitor of the DNA Restriction System of Escherichia coli*

Kai-Keung Mark‡ and F. William Studier
From the Biology Department, Brookhaven National Laboratory, Upton, New York 11973

The gene 0.3 protein of bacteriophage T7 prevents the DNA restriction system of Escherichia coli from interfering with T7 infection. A mutant strain of T7 that greatly overproduces the 0.3 protein has been constructed and used for purification of this protein. The 0.3 protein was found to be extremely acidic and can be separated from virtually all other proteins of the infected cell by chromatography on DEAE-cellulose. Residual contaminating proteins and nucleic acids can be removed by gel filtration, but an even simpler final purification is possible, because under appropriate conditions the 0.3 protein is soluble in high concentrations of ethanol. Thus, a simple, essentially two-step purification can produce about 50 mg of pure 0.3 protein from 30 liters of culture. The purified protein appears to be a dimer of identical subunits. As expected from its known function during infection, the purified 0.3 protein inhibits the nuclease and ATPase activities of partially purified Eco B, the DNA restriction enzyme of E. coli B, but it does not interfere with several different type II restriction endonucleases tested. The inhibition of Eco B appears to require stoichiometric rather than catalytic amounts of 0.3 protein.

The first protein made by bacteriophage T7 after infection of Escherichia coli is responsible for overcoming host restriction (Studier, 1975). This protein, the product of T7 gene 0.3, prevents both degradation and modification of T7 DNA by the restriction-modification complex of E. coli. The equivalent protein of the related bacteriophage T3 is the S-adenosylmethionine-cleaving enzyme (Studier and Movva, 1976), but no AdoMet hydrolase activity nor any other enzymatic activity has been found to be associated with the 0.3 protein of T7.

We have purified the T7 0.3 protein in order to study its mechanism of action, but also because information about its amino acid sequence was needed in order to study the effects of T7 mutations that affect initiation of synthesis of the 0.3 protein (Dunn et al., 1978). Purification was greatly facilitated by the use of T7 mutants that overproduce the 0.3 protein and that allow it to be labeled specifically, so that initially the protein could be followed through purification simply by monitoring radioactivity, and ultimately simply by gel electrophoresis and staining. This paper reports purification and initial characterization of the T7 0.3 protein; the accompanying paper (Dunn et al., 1981) reports its amino acid sequence and the nucleotide sequence of its mRNA; and a subsequent paper will report details of the interaction between the purified 0.3 protein and purified Eco B and Eco K, the restriction endonucleases of E. coli B and K-12 strains.

EXPERIMENTAL PROCEDURES

Phage and Bacteria—The strain of T7 used for specific 35S labeling and for production of 0.3 protein was the triple mutant H3.am193.LG3, whose individual mutations are described in Studier (1969) and Studier et al. (1979). E. coli C1757, used to propagate the triple mutant, and E. coli B or B834, used for production of 0.3 protein or for specific labeling with [35S]methionine, have been described (Studier, 1975); C1757 and B834 lack an active restriction-modification system, and C1757 carries the supD amber suppressor.

Type II Restriction Endonucleases—Hpa I, Hpa II, HincII, and Hae III were purchased from Bethesda Research Laboratories or New England Biolabs, Beverly, MA.

Gel Electrophoresis—Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was done essentially as described (Studier, 1973). Samples were heated for 2 min in a boiling water bath before electrophoresis. Slab gels contained a linear gradient of acrylamide, from 10–20%, with a 5% stacking gel. The Tris chloride/glycine stacking buffer system was used, and electrophoresis was at 150–200 V until the bromphenol blue tracking dye reached the bottom of the gel, usually 2 h or so. Proteins were visualized by staining the gel with Coomassie blue (Maizel, 1971).

DNA was analyzed by electrophoresis in a horizontal 0.5% agarose slab gel essentially as described (McDonell et al., 1977) or in an improved apparatus. Electrophoresis was in 40 mM Tris, 20 mM acetic acid, 2 mM Na2EDTA at room temperature for 2 h at 5 V/cm or overnight at 0.75 V/cm. The DNA was visualized by its fluorescence in ethidium bromide solution (0.5 µg/ml in H2O) (Sharp et al., 1973). The figures showing gel patterns are oriented so that the origin of electrophoresis is at the top.

35S-labeled 0.3 Protein—The H3.am193.LG3 triple mutant was used to prepare specifically labeled 0.3 protein for use in working out purification procedures. Six ml of UV-irradiated E. coli B (Studier and Maizel, 1969) were infected and labeled with [35S]methionine (20 µCi/ml; 100 ng/ml) between 2 and 15 min after infection. The labeled cells were collected by centrifugation, resuspended in buffer, and frozen. Virtually all of the label incorporated into protein was in 0.3 protein, as shown by electrophoretic analysis. The result of a similar labeling experiment is shown in the third lane from the right in Fig. 2, but the sample actually used for purifying 0.3 protein had an even greater fraction of the total 35S in 0.3 protein than did the sample of Fig. 2.) When samples of these specifically labeled cells were added to batches of infected cells used to prepare 0.3 protein, the 0.3 protein could be followed through purification procedures simply by monitoring 35S.

Large Scale Preparation of 0.3 Protein—The following procedure produces approximately 30 mg of pure 0.3 protein: 32 liters of E. coli B or B834 are grown at 30°C with vigorous aeration in tryptone broth to which the components of M9 medium have been added (Studier, 1969). When the concentration of bacteria reaches 5 x 109 to 1010/ml,
4 liters of phage lysate are added, typically containing $2 \times 10^m$ infective Pl.am.183, LiG3 particles/ml, to give a final concentration of $2 - 4$ particles/bacterium. The concentration of viable bacteria is measured immediately before and 5 min after infection, to estimate the efficiency of infection. Typically, more than 90% of the cells are infected. Thirty min after infection, the culture is poured into a 50-liter container filled 1/3 full of ice cubes, which is more than enough ice to chill the entire culture to 0°C. The infected cells are then collected by centrifugation through a Sorvall T-28 continuous flow rotor at 18,000 rpm at flow rates as high as 1400 ml/min.

The cell paste, typically 65-75 g, is suspended in 200 ml of 50 mM Tris chloride, pH 8, 10 mM Na,EDTA, 4% glycerol. An attempt is made to disperse the cells, but usually the paste is very sticky and does not disperse well. The material is frozen and thawed 3 times to break open the cells, and 2 ml of 1 M MgSO$_4$ plus 1 ml of pancreatic DNase (Worthington DP, 2 mg/ml in H$_2$O) are added to the viscous preparation. The suspension is shaken in a 1-liter flask at the maximum speed of a New Brunswick model 75 gyratory water bath shaker at 30°C for 30 min, by which time the solution has lost its high viscosity and has become well dispersed. The volume is measured and the solution adjusted to 0.3 M NH$_4$Cl by adding solid NH$_4$Cl. The extract is then centrifuged 15 min at 15,000 rpm in a Sorvall SS-34 rotor and the supernatant centrifuged 1 h at 40,000 rpm in a Beckman 42.1 rotor, both at 4°C.

The supernatant from the second centrifugation is immediately passed through a 35-ml DEAE-cellulose column at room temperature. The DEAE-cellulose (Whatman DE52) is prepared by settling twice in 0.3 M NH$_4$Cl, 20 mM Tris chloride, pH 8, 4% glycerol, packing into a 50-ml plastic syringe, and washing with about 120 ml of the same solvent. The flow rate is typically 5 ml/min. It is important to load the extract onto the column precipitately after centrifugation, because the material slowly comes out of solution and can stop the flow through the column. (If this happens, the column is simply unpacked, settled in the same solvent, and repacked.) After the crude extract has passed through, the column is washed with about 200-250 ml of the same solvent and then eluted with a 400-ml linear gradient of 0.3-1 M NH$_4$Cl in 20 mM Tris chloride, pH 8, 4% glycerol, collecting 20-ml fractions. The peak of 0.3 protein is located by analyzing 25 μl of each fraction by gel electrophoresis in the presence of sodium dodecyl sulfate (see Fig. 1). The peak is typically in fractions 8-12 of the 20 fractions from the gradient, and more than 95% of the protein in the pooled peak fractions is 0.3 protein. Analysis of 25 μl of each gradient fraction overloads the gel for 0.3 protein at the peak but allows the minor proteins to be visualized.

Contaminating nucleic acids and proteins can be removed by gel filtration (Bio-Gel A-0.5m and Sephadex G-100 have been used). However, in most cases the 0.3 protein is simply precipitated directly from the DEAE-cellulose column fractions with an equal volume of 10% trichloroacetic acid and redissolved in 95% ethanol. The contaminating material is then insoluble in the ethanol, and after two cycles of precipitation and dissolution, the 0.3 protein is essentially free of contaminating nucleic acids and proteins. The 0.3 protein can be recovered from the precipitate by precipitation in 1.5-2 volumes of 10% trichloroacetic acid and then dissolved in neutral aqueous buffer. The purified 0.3 protein has been stored in neutral aqueous buffer at 4°C or frozen at −20°C, or in ethanol solution at −20°C; its specific activity for inhibition of the nuclease activity of Eco B seems to be quite stable under these conditions.

**Estimates of Molecular Weight**—The molecular weight of the native 0.3 protein was estimated relative to proteins of known molecular weight by gel filtration chromatography or by sucrose gradient centrifugation. Gel filtration was on a Sephadex G-100 superfine column (0.5 × 55 cm) which was equilibrated and eluted with 1 mM NaCl, 20 mM Tris chloride, pH 7.5, at 4°C. Centrifugation was at 20°C for 16 h at 55,000 rpm in a Beckman SW 65 rotor in a 5-20% sucrose gradient containing the same buffer as above or containing 70 mM Tris chloride, 6 mM MgCl$_2$, pH 8, the buffer used in nuclease and ATPase assays of Eco B. The marker proteins (molecular weight of the supernatant of E.coli B mutant T7 RNA polymerase (Shapiro et al., 1967; Weber and Osborn, 1969) were ribonuclease A (66,296), ovalbumin (43,000), chymotrypsinogen A (25,660), and pancreatic RNase A (13,690). The positions of the individual proteins after fractionation were determined by analyzing a portion of each fraction by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, which itself provided an estimate of the subunit size of the 0.3 protein (Shapiro et al., 1967; Weber and Osborn, 1969).

**Assays for Nuclease and ATPase Activities of Eco B**—Most experiments to test the effect of 0.3 protein on the nuclease and ATPase activities of the restriction endonuclease of E. coli B (Eco B) used a preparation of E. coli B that had been purified through the DEAE-cellulose concentration step (Eskin and Linn, 1972), the kind gift of S. Linn. This material had a protein concentration of about 2 mg/ml, its nuclease activity was strictly dependent on added ATP and S-adenosylmethionine (see Fig. 4), and 1 μl would make about 2 × 10$^7$ double-strand cuts in T7 DNA. Assuming that each active Eco B makes one double-strand cut in DNA and that the native M, = ~250,000,500,000 (Eskin and Linn, 1972), active Eco B would be expected to represent only perhaps 1-2% of the total protein in this preparation.

**RESULTS**

**T7 Strains That Overproduce 0.3 Protein**—During infection of E. coli by wild type T7 (at 30°C), the 0.3 protein is made at a relatively high rate between about 4 and 8 min after infection, but its synthesis stops abruptly about the time the late proteins begin to be made (Studier, 1972; see also Fig. 2). Active synthesis of the 0.3 protein can be prolonged and accelerated by use of appropriate T7 mutants. Mutants defective in gene I, the T7 RNA polymerase, do not shut off synthesis of the 0.3 protein at the usual time but continue to synthesize it actively for at least 20 min (Studier, 1972). The shut off that occurs in wild type T7 is apparently due to competition for the protein synthetic machinery by the late T7 messenger RNAs produced by the T7 RNA polymerase (Strome and Young, 1978). However, even in gene I mutants the rate of synthesis of the 0.3 protein slowly declines as the 0.3 mRNA

---

Fig. 1. Fractionation of 0.3 protein by DEAE-cellulose chromatography. Samples of individual column fractions were analyzed by electrophoresis in presence of sodium dodecyl sulfate, which itself provided an estimate of subunit size of the 0.3 protein (Shapiro et al., 1967; Weber and Osborn, 1969).
decays, because the action of gene 0.7, another early gene, shuts off synthesis of the 0.3 mRNA about 4 min after infection (Brunovskis and Summers, 1972; Rothman-Denes et al., 1973). Double mutants lacking both gene 0.7 and 1 functions continue to produce 0.3 mRNA, and the rate of synthesis of 0.3 protein remains very strong and even increases over a period of at least 30 min.

We have used a triple mutant H3,am193,LG3 for production of 0.3 protein; am193 is an amber mutation in gene 1, and H3 and LG3 are deletions that eliminate all of the other early proteins except the 0.3 and 0.4 proteins, which are specified by the 0.3 mRNA. (For a detailed description of these deletions and of the early region of T7 DNA, see Studier et al. (1979)) The time course of protein synthesis by the triple mutant after infection of the nonsuppressing host E. coli B is compared with that of wild type T7 in Fig. 2. As noted above, synthesis of 0.3 protein during the wild type infection stops abruptly about 8 min after infection, at the same time synthesis of host proteins stops; but with the triple mutant neither host protein synthesis nor 0.3 protein synthesis shut off, and the 0.3 protein becomes the most actively synthesized protein in the cell. The 0.4 protein, a small protein of unknown function, is made in relatively small amounts and is not easily distinguished in the gel patterns of Fig. 2. The 0.3 protein is actively synthesized until at least 32 min after infection and would probably continue to be synthesized indefinitely, but cultures are harvested 30 min after infection because gradual lysis seems to begin around this time (perhaps due to accumulation of small amounts of T7 late proteins).

**Purification of 0.3 Protein**—An experiment to work out procedures for purifying 0.3 protein used 3.6 g of cells collected from 2 liters of E. coli B 30 min after infection with the triple mutant H3,am193,LG3. The infected cells were suspended in 10 ml of buffer, and a crude extract was prepared essentially as described for the large scale purification under “Experimental Procedures,” except that 35S-labeled infected cells collected from 0.6 ml of UV-treated culture (containing 640,000 cpm, almost entirely in 0.3 protein) were added before the freezing and thawing, as was lysozyme (100 μg/ml, to ensure good lysis of the UV-treated cells). The concentration of 35S in the supernatant after the centrifugation at 40,000 rpm was 83% that in the initial lysate, indicating that virtually all of the 0.3 protein is to be found in the supernatant after this step.

The 40,000 rpm supernatant (8 ml) was adjusted to 1 M NaCl, applied to a Bio-Gel A-0.5 m column (5 x 76 cm) that had been equilibrated at 4°C with 1 M NaCl, 20 mM Tris chloride, pH 8, 1 mM mercaptoethanol, and eluted with the same buffer. A single peak of 35S, containing about 60% of the input counts, was observed at slightly more than twice the void volume of the column, and no other peak of label was observed. The column fractions were also assayed for ability to degrade T7 DNA in the presence of Mg2+, S-adenosylmethionine, and ATP, the conditions needed for Eco B activity. No nuclease activity was found in fractions near the void volume, where Eco B might be expected to elute, but a peak of nuclease activity was centered in the fractions just behind the peak of 35S, near 2.5 void volumes. This activity presumably represents some nuclease other than Eco B. Gel electrophoresis of samples from the fractions through the peak of 35S showed a prominent band at the position expected for the 0.3 protein, but less than 10% of the total protein in the peak fractions was estimated to be in this band.

The fractions containing the peak of 35S were pooled, dialyzed against 20 mM Tris chloride, pH 7.5, 0.1 mM dithiothreitol, 5% glycerol, loaded onto a 5-ml column of DEAE-cellulose, and eluted with a 100-ml linear gradient of 0-0.5 M NH4Cl in the same buffer. The 35S was efficiently adsorbed to the column, but surprisingly, very little of it eluted; and what little did come out was in the final fraction of the gradient. However, washing the column with buffer containing 1 M NH4Cl eluted the remainder of the 35S (about 80% recovery of input counts). Virtually all of the nuclease activity eluted within the gradient fractions and therefore was separated from the 0.3 protein. The peak fractions of 35S were pooled and chromatographed on a second DEAE-cellulose column, this time with a gradient from 0.2-1 M NH4Cl. The 35S eluted in a single peak around 0.5-0.6 M NH4Cl. No nuclease activity could be detected in the peak fractions even after overnight incubation at 37°C, and the ratio of absorbance at 260 to 280 nm was 1.61, suggesting that the protein was substantially free of nucleic acid. Gel electrophoresis of samples from the peak fractions showed that over 99% of the protein, as measured by Coomassie blue staining, migrated as a single band at the position expected for the 0.3 protein.

These results led to development of the purification procedure described under “Experimental Procedures.” Since very few E. coli proteins adsorb to DEAE-cellulose in 0.3 M NH4Cl, a relatively small column can be used to selectively adsorb all of the 0.3 protein from a crude extract without becoming overloaded. As shown in Fig. 1, this simple procedure is very selective for purifying 0.3 protein. Small amounts of a few other proteins tail into the peak of 0.3 protein, but only one protein appears to bind to the column as tightly as the 0.3 protein; and in extracts from the overproducing strain, this protein appears to be present in less than 1% of the amount of 0.3 protein.

![Fig. 2. Protein synthesis after infection of E. coli B by wild type T7 or by the H3,am193,LG3 triple mutant. Samples were labeled and analyzed by gel electrophoresis as described under "Experimental Procedures." All of the samples except the second and third lanes from the right represent 50 μl of culture labeled for 4 min at 30°C with 1 μCi of [35S]methionine (100 ng/ml). The two outside 30 lanes were from uninfected cells; the second to ninth lanes from the left were from culture infected with wild type T7, labeled between 0-4, 4-8, 8-12, 12-16, 16-20, 20-24, 24-28, and 28-32 min after infection; and the next eight lanes were from culture infected with H3,am193,LG3, labeled the same times after infection. The second lane from the right represents 50 μl of UV-treated uninfected culture that was labeled for 13 min with 2 μCi of [35S]methionine (100 ng/ml); the third lane from the right represents 50 μl of UV-treated culture infected with H3,am193,LG3 and labeled in the same way between 2-15 min after infection. The most heavily labeled band in the third lane from the right is the 0.3 protein; other T7 proteins may be identified by reference to protein patterns in Studier (1972).](image-url)
Over 95% of the protein present in the pooled peak fractions from DEAE-cellulose chromatography of a crude extract appears to be 0.3 protein. However, the ratio of absorbance at 280 to 290 nm for this material is typically only 0.55-0.75, indicating that substantial amounts of nucleic acid remain.

The 0.3 protein can be separated from contaminating nucleic acids and proteins by gel filtration, but a much simpler method was discovered. In preparing column fractions for amino acid analysis, we wished to remove the large amount of NH4Cl, which would interfere with the analysis. To do this, the protein was precipitated with an equal volume of 10% trichloroacetic acid. When the pellet was washed with 95% ethanol, it was found that the 0.3 protein in the pellet dissolved in the ethanol. The nucleic acids and contaminating proteins, on the other hand, remained insoluble and could be removed by centrifugation. The 0.3 protein could be recovered from the ethanol solution by precipitation with 1.5-2 volumes of 10% trichloroacetic acid and would redissolve in aqueous buffers. Two cycles of precipitation and ethanol extraction typically produce a pure preparation of 0.3 protein that has a 280/260 ratio of about 2.0, which does not change upon further precipitation and extraction.

No exhaustive study of the solubility of 0.3 protein in ethanol was made, but it was noted that the protein precipitates upon neutralization of the 95% ethanol solution. On the other hand, the 0.3 protein remains soluble when 2 volumes of ethanol are added to the neutral column fractions from the NH4Cl gradient. This can be useful for removing contaminants when the 0.3 protein is a minor component, as for example with mutants that produce it in small amounts. In such cases, direct precipitation of the column fractions with trichloroacetic acid would produce a pellet that is mostly insoluble in ethanol, and extraction of the small amount of 0.3 protein is inefficient. The nature of the cation apparently has some effect on solubility, as addition of 2 volumes of ethanol to fractions from a column that had been eluted with NaCl rather than NH4Cl precipitated a considerable fraction of the 0.3 protein.

Although precipitation with trichloroacetic acid and dissolving in 95% ethanol would not be considered gentle treatments for a protein, they seem to have little if any effect on the 0.3 protein. The 0.3 protein is readily soluble in aqueous buffer after such treatment, and its apparent molecular weight, as estimated by sucrose gradient centrifugation, is unchanged. Furthermore, this treatment has no effect on the specific activity of the 0.3 protein for inactivating the nuclease activity of Eco B.

**Physical Properties of the 0.3 Protein**—As shown in the accompanying paper, the 0.3 protein contains 116 amino acids, of which 34 are acidic and 6 are basic. This large proportion of acidic residues explains the high affinity of the 0.3 protein for DEAE-cellulose. The formula weight for a monomer of 0.3 protein is 13,678.

The molecular weight of 0.3 protein monomer was originally estimated by gel electrophoresis in the presence of sodium dodecyl sulfate to be 8,700 (Studier, 1972), but has subsequently been estimated to be closer to 14,000 (Ratner, 1974; Strome and Young, 1978). In the experiment of Fig. 3, the 0.3 protein migrated slightly ahead of RNase A, which has almost exactly the same formula weight as 0.3 protein. However, in a continuous Tris chloride buffer system the 0.3 protein ran considerably ahead of RNase A, at a position that would correspond to $M_r = 11,000$. Molecular weight estimates by gel electrophoresis in the presence of sodium dodecyl sulfate are known to depend to some extent on which gel system and which marker proteins are used (Shapiro et al., 1967; Studier and Maizel, 1969).

To determine whether native 0.3 protein is present in solution as a monomer or higher aggregate, samples of 0.3 protein were analyzed relative to proteins of known molecular weight by centrifugation through sucrose gradients and by chromatography on a Sephadex G-100 column, as described under "Experimental Procedures." Results of one such experiment are shown in Fig. 3. In all cases, the 0.3 protein was found in a position corresponding to a $M_r = 28,000-30,000$, indicating that the 0.3 protein exists as a dimer in aqueous solution both at high ionic strength and under the conditions used to assay Eco B activity. No difference was observed between preparations of 0.3 protein whether or not they had been subjected to precipitation by trichloroacetic acid.

**Activity of Purified 0.3 Protein**—Since the 0.3 protein acts in vivo to prevent restriction of T7 DNA by the E. coli restriction system, we expected that purified 0.3 protein would prevent degradation of T7 DNA by the purified restriction endonuclease of E. coli. This was tested first with crude preparations of Eco B (dialyzed ammonium sulfate fraction, as described by Linn and Arber, 1968) and subsequently with the partially purified sample described under "Experimental Procedures." During purification of 0.3 protein, fractions from both Bio-Gel A-0.5m and DEAE-cellulose columns were tested; in both cases a peak of inhibitory activity co-chromatographed exactly with the peak of 0.3 protein found by 280/260, and by gel electrophoresis, and no inhibitory activity was observed in other column fractions. Therefore, the 0.3 protein does have the ability to inhibit Eco B activity in vitro, and seems to be the only material with this activity in the extract of infected cells.

**Fig. 3.** Sedimentation velocity and gel electrophoresis of 0.3 protein relative to protein standards. 200 μl of a solution containing 40 μg each of bovine serum albumin, ovalbumin, chymotrypsino- gen, RNase A, and 0.3 protein were layered atop a 5-ml 5-20% sucrose gradient containing 70 mM Tris chloride, 6 mM MgCl2, pH 8, and centrifuged for 16 h at 55,000 rpm at 20°C in an SW 65 Ti rotor in a Beckman centrifuge. Sixteen approximately equal fractions were collected by dripping from the bottom of the punctured tube, and 25 μl of each were analyzed by gel electrophoresis in the presence of sodium dodecyl sulfate. The lanes, from left to right, contain the five individual proteins in the order given above (1 μg each), and the 16 fractions in order from the bottom of the tube to the top. Purification of the 0.3 protein used in this experiment had included precipitation with trichloroacetic acid and dissolution in ethanol, but equivalent results were obtained with 0.3 protein that had not been subjected to these treatments.
Using purified 0.3 protein, we find that inhibition of Eco B is stoichiometric rather than catalytic. The concentration of 0.3 protein was measured by amino acid analysis of a stock solution that was greater than 99% pure, and the concentration of active Eco B was estimated as described under "Experimental Procedures." Typical results are shown in Fig. 4 and Table I. The first experiment of Fig. 4 shows that, as expected, the nuclease activity of this preparation of Eco B was completely dependent on added S-adenosylmethionine and ATP, and was essentially completely inhibited by an excess of 0.3 protein. The second experiment (Fig. 4 and Table I) shows the effects of different concentrations of added 0.3 protein on the nuclease and ATPase activities of the Eco B. Based on the estimated concentrations of the two proteins, 10–20 dimer molecules of 0.3 protein were needed per active Eco B to prevent cleavage of T7 DNA, and the same concentration of 0.3 protein was needed to inhibit ATPase activity. The inability of low molar ratios of 0.3 protein to active Eco B molecules to prevent restriction suggests that the 0.3 protein inactivates Eco B by binding to it. The possibility that a large fraction of the 0.3 protein molecules were inactive seems unlikely, as several different preparations of 0.3 protein purified in different ways were all found to have about the same specific activity. The need for a large excess of 0.3 protein might reflect the binding affinity or the kinetics of inhibition under these conditions, a possible need for binding more than 1 molecule of 0.3 protein/molecule of Eco B for complete inhibition, the possibility that 0.3 protein may bind to an excess of inactive Eco B molecules that could be present in the preparation, or some combination of these or other possibilities. More detailed studies of the interaction between 0.3 protein and the Eco B and Eco K restriction enzymes will be reported in another paper.

**Effect of 0.3 Protein on Type II Restriction Endonucleases—**Inhibition of the nuclease activity of Eco B by the 0.3 protein is specific for Eco B and not for the T7 DNA substrate. Thus, 0.3 protein prevents nuclease activity by Eco B against unmodified λ DNA equally as well as against T7 DNA but has little if any effect on the nuclease activities of the type II restriction endonucleases Hpa I, Hpa II, HincII, or HaeIII even at concentrations of 0.3 protein 100 times higher than needed to inhibit Eco B (results not shown).

**DISCUSSION**

The 0.3 protein inhibits the nuclease and ATPase activities of partially purified Eco B, apparently by direct binding to the Eco B enzyme complex. In contrast to the 0.3 protein of T7, the protein responsible for preventing host restriction during infection by the related bacteriophage T3 has the ability to hydrolyze S-adenosylmethionine (Studier and Movva, 1976; Gefter et al., 1966), a cofactor that is required for the restriction activity of both Eco B and Eco K enzymes. However, Sporrel et al. (1979) have reported that the anti-restriction activity of the T3 AdoMet hydrolase against purified Eco K is due to a direct interaction with the restriction endonuclease rather than to its AdoMet hydrolase activity as such. Thus, the primary means of preventing restriction may be very similar for the proteins of the two related phages even though the proteins themselves appear to be physically and genetically unrelated. The unrelatedness of the two proteins is shown by the following observations: the T7 protein has not been found to have any AdoMet hydrolase activity, either in crude extracts (Gefter et al., 1966; Studier and Movva, 1976) or as the purified protein (this work, data not shown); the T7 protein is so acidic that it does not elute from DEAE-cellulose until 0.5–0.6 M NH₄Cl (this work), whereas the T3 AdoMet hydrolase passes through DEAE-cellulose in 20 mM Tris chloride buffer with no added salt (Sporrel and Herrlich, 1979); the two proteins have little if any immunological cross reaction (Sporrel et al., 1979; Sporrel and Herrlich, 1979); and the DNA segments that code for these two proteins have little if any homology (Davis and Hyman, 1971). How two such different proteins came to have the same role during the infection of related bacteriophages is an interesting evolutionary question.

Before the role of 0.3 protein in overcoming host restriction was known, it was suggested that the 0.3 protein may have a role in control of transcription or translation after T7 infection. Ratner (1974) reported that the 0.3 protein binds to an affinity column containing immobilized E. coli RNA polymerase, and

**TABLE I**

**Inhibition of the ATPase activity of Eco B by added 0.3 protein**

A portion of these reaction mixtures was also assayed for nuclease activity (Fig. 4). The ATPase assay (Eskin and Linn, 1972) is based on release of α-32P from γ-32PATP, as detected by loss of ability to adsorb to Norit. Release of all of the 32PO₄ would have given 5710 cpm.

| Reaction mixture | 0.3 protein added | Approximate molar ratio 0.3 to dimeric active Eco B | 32PO₄ released from γ-32P-ATP | Relative ATPase activity |
|------------------|-------------------|---------------------------------------------------|-------------------------------|--------------------------|
| Blank, no Eco B  | 0.3 ng             | 480                                               | 480                           | 0                        |
| Complete         | 225 ng             | 3450                                              | 3450                          | 100                      |
| Complete         | 22.5 ng            | 470                                               | 470                           | 0                        |
| Complete         | 7.5 ng             | 550                                               | 550                           | 2                        |
| Complete         | 2.5 ng             | 940                                               | 940                           | 15                       |
| Complete         | 0.83 ng            | 2130                                              | 2130                          | 56                       |
| Complete         | 0.28 ng            | 3730                                              | 3730                          | 109                      |

* K.-K. Mark and F. W. Studier, unpublished observations.
Herrlich et al. (1974) proposed that some protein that maps near the left end of the early region of T7 DNA is a "translational-repressor" that shuts off the synthesis of host proteins after infection. However, the protein patterns of Fig. 2 show that neither the 0.3 nor 0.4 proteins could be acting either to affect transcription or translation in vivo, as the synthesis of host proteins continues at essentially the preinfection rate for at least 32 min even in the presence of a vast overproduction of the 0.3 and (presumably) 0.4 proteins. Perhaps the binding of 0.3 protein to RNA polymerase observed by Ratner is nonspecific and similar to the binding of 0.3 protein to DEAE-cellulose. We have not tried to analyze any interactions of purified 0.3 protein with purified E. coli RNA polymerase.

REFERENCES
Brunovskis, I., and Summers, W. C. (1972) Virology 50, 322-327
Davis, R. W., and Hyman, R. W. (1971) J. Mol. Biol. 62, 287-301
Dayhoff, M. O. (1972) Atlas of Protein Sequence and Structure, Vol. 5, National Biomedical Research Foundation, Silver Spring, Maryland
Dayhoff, M. O. (1976) Atlas of Protein Sequence and Structure, Vol. 5, Suppl. 2, National Biomedical Research Foundation, Silver Spring, Maryland
Dunn, J. J., Buzash-Pollert, E., and Studier, F. W. (1978) Proc. Natl. Acad. Sci., U. S. A. 75, 2741-2745
Dunn, J. J., Elzinga, M., Mark, K.-K., and Studier, F. W. (1981) J. Biol. Chem. 256, 2579-2585
Eskin, B., Lautenberger, J. A., and Linn, S. (1973) J. Virol. 11, 1020-1023
Eskin, B., and Linn, S. (1972) J. Biol. Chem. 247, 6183-6191
Geifer, M., Hausmann, R., Gold, M., and Hurwitz, J. (1969) J. Biol. Chem 241, 1995-2006
Herrlich, P., Rahmsdorf, H. J., Pai, S. H., and Schweiger, M. (1974) Proc. Natl. Acad. Sci., U. S. A. 71, 1088-1092
Linn, S., and Arber, W. (1968) Proc. Natl. Acad. Sci., U. S. A. 59, 1300-1306
Maizel, J. V., Jr. (1971) Methods Virol. 5, 179-246
McDonell, M. W., Simon, M. N., and Studier, F. W. (1977) J. Mol. Biol. 110, 119-146
Ratner, D. (1974) J. Mol. Biol. 88, 373-383
Roberts, R. J. (1978) Gene 4, 183-189
Rothman-Denes, L. B., Muthukrishnan, S., Haselkorn, R., and Studier, F. W. (1973) in Virus Research (Fox, D. F., and Robinson, W. S., eds) pp. 227-239, Academic Press, New York
Sharp, P. A., Sugden, B., and Sambrook, J. (1973) Biochemistry 12, 3065-3063
Shapiro, A. L., Viñuela, E., and Maizel, J. V., Jr. (1967) Biochem. Biophys Res. Commun. 28, 815-820
Spoerel, N., and Herrlich, P. (1979) Eur. J. Biochem. 95, 227-233
Spoerel, N., Herrlich, P., and Bickle, T. A. (1979) Nature 278, 30-34
Strome, S., and Young, E. T. (1978) J. Mol. Biol. 125, 75-93
Studier, F. W. (1969) Virology 38, 562-574
Studier, F. W. (1972) Science 176, 367-376
Studier, F. W. (1973) J. Mol. Biol. 79, 237-248
Studier, F. W. (1975) J. Mol. Biol. 94, 283-295
Studier, F. W., and Maizel, J. V., Jr. (1969) Virology 38, 575-586
Studier, F. W., and Movva, N. R. (1976) J. Virol. 19, 136-145
Studier, F. W., Rosenberg, A. H., Simon, M. N., and Dunn, J. J. (1979) J. Mol. Biol. 135, 917-937
Weber, K, and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412