T3 and T7 Bacteriophage Deoxyribonucleic Acid-Directed Enzyme Synthesis In Vitro

PETER HERRLICH AND MANFRED SCHWEIGER¹

The Rockefeller University, New York, New York 10021

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The T3 phage enzymes *S*-adenosyl methionine cleaving enzyme and lysozyme and the T7 lysozyme were synthesized in a deoxyribonucleic acid (DNA)-dependent, cell-free system derived from uninfected *Escherichia coli*. The data presented suggest that these enzymes are encoded in that portion of the DNA which is transcribed early after infection.

Three viral proteins are made in the early period following infection of *Escherichia coli* with phage T7 (8). Only one of these proteins has been assigned a certain function, which is to control the synthesis of most of the phage proteins (8, 9). The functions of the other proteins are unknown, but one can presume that these "early" proteins are of special importance for phage development. Therefore, we have investigated the possible roles of these proteins, using an in vitro system (5) capable of synthesizing enzymes under the direction of deoxyribonucleic acid (DNA).

In this paper, we present results which suggest that one of the two unknown early T7 proteins is lysozyme, and that in the closely related phage T3 two of the unknown early proteins are lysozyme and the *S*-adenosyl methionine cleaving enzyme.

MATERIALS AND METHODS

T3 phage stock came from Walter Sauerbier, and T7 phage was kindly donated by F. William Studier. DNA was prepared as described previously, as was the cell-free system from *E. coli* 514 (3).

Determination of enzyme activities. Lysozyme was estimated by excorporation of radioactive material from labeled cell walls (6). The *S*-adenosyl methionine cleaving enzyme was assayed by a thin-layer chromatographic method. Samples (0.002 ml) of protein synthesis reaction mixtures were combined with a solution of ¹⁴C-CH₃-labeled *S*-adenosyl methionine in tris(hydroxymethyl)aminomethane (0.01 M, pH 7.0). They were incubated at 30°C for different periods of time, and then 5-μl portions of the reaction mixture were spotted on cellulose sheets (Eastman chromatogram sheet 6065, Distillation Products Industries). The chromatograms were developed with 64% ethyl alcohol in water, and the radioactivity was counted in a Radio Scanner (Berthold, Varian Aerograph), or the spots of *S*-adenosyl methionine and thiomethyl

adenosine were located with an ultraviolet lamp, cut out, and counted in a liquid scintillation spectrometer. The radioactivity in the splitting-product spot was a measure of the enzyme and was expressed as a percentage of the total radioactivity in the chromatogram.

RESULTS

T7 and T3 DNA-dependent synthesis of lysozyme. Lysozyme synthesis was directed by added T7 or T3 DNA (Table 1). The appearance of this activity was dependent on protein synthesis. This was shown by its inhibition by specific inhibitors of transcription and translation, or by the low control values obtained with samples that had not been incubated at 37°C. The amount of lysozyme synthesized under the direction of T3 and T7 DNA was 30 to 40 times larger than the amount of T4 DNA-dependent lysozyme synthesized under comparable conditions (6).

Synthesis of *S*-adenosyl methionine cleaving enzyme. In addition to causing lysozyme synthesis, T3 DNA caused the appearance of an *S*-adenosyl methionine cleaving activity (Fig. 1). This was also due to de novo enzyme synthesis, as shown by similar controls (Fig. 2). The amount of enzyme synthesized was truly remarkable (Fig. 2). A portion of 0.002 ml of the incubation mixture contained enough enzyme synthesized in vitro to split 50% of the added *S*-adenosyl methionine in 50 min at 30°C. It should be mentioned that the enzyme reactions were performed under sub-optimal conditions. At 30°C, the enzyme reaction was almost linear for more than 90 min (Fig. 3). When the substrate concentration was high, a second radioactive peak appeared in the chromatogram. This product might be γ-aminobutyrolactone, an intermediate in the enzyme reaction (2), or it could be a result of splitting the substrate into adenosine and methionine. Indeed,

¹ Present address: Max-Planck-Institut für Molekulare Genetik, Berlin 33, Germany.

TABLE 1. *Lysozyme synthesis in response to T7 DNA*

Expt	Conditions	Lysozyme activity ^a
I	Without DNA	1,425
	T3 DNA	59,200
	T3 DNA, without 37 C incubation	541
	T3 DNA, chloramphenicol (100 µg/ml)	661
II	T3 DNA	79,400
	T3 DNA, rifampicin (20 µg/ml)	518
	T3 DNA, actinomycin D (28 g/ml)	484
	T3 DNA, chlortetracycline (100 µg/ml)	1,347
	T3 DNA, fusidic acid (10 ⁻³ M)	1,062
III	T3 DNA, neomycin B (200 µg/ml)	1,540
	T7 DNA	56,400
	T7 DNA, chloramphenicol (100 µg/ml)	720
	T7 DNA, actinomycin D (35 µg/ml)	1,305
	T7 DNA, actinomycin D (150 µg/ml)	780
	T7 DNA, rifampicin (100 µg/ml)	740

^a Protein synthesis was carried out as described previously (5). After incubation for 40 min at 37 C, lysozyme activities were measured as described (5). Results are expressed as counts per minute per hour per 250 µg of rib. Counts per minute represent 0.2 ml of the supernatant. In all experiments, the magnesium concentration was 11 mM. The filter blank control value was 500 counts/min and is not subtracted.

methionine has an R_F very similar to that of the unknown radioactive peak when chromatographed with the same ethyl alcohol solvent. In addition, a substance with ultraviolet absorption and with an R_F similar to that of adenosine was detected in our chromatograms. The cleaving product at low concentrations of *S*-adenosyl methionine co-chromatographed with thio-methyl-adenosine, which is the natural product of the reaction catalyzed by the enzyme *in vivo*.

DISCUSSION

Soon after infection of *E. coli* with phage T7, three proteins are made which have molecular weights of 95,000 to 100,000, 40,000 to 45,000, and 15,000 to 20,000 daltons (8). The protein with the molecular weight of about 100,000, the gene 1 product, has a positive control function for the synthesis of the late phage proteins. Amber mu-

FIG. 1. T3 DNA-dependent synthesis of *S*-adenosyl methionine cleaving enzyme. Separation of *S*-adenosyl methionine and reaction products by thin-layer chromatography. Samples (50 µliters) were incubated, under conditions of protein synthesis (6), either with T3 DNA (for 1a and 1b) or as a control with T4 DNA (for 1c). Samples of 10 µliters were used for the enzyme reaction. The samples contained in a total volume of 22 µliters: (a) protein synthesis mixture with T3 DNA, 5.9 nmoles of ¹⁴C-methyl-*S*-adenosyl methionine (6.78 Ci/mole); (b) protein synthesis mixture with T3 DNA, 25.9 nmoles of ¹⁴C-methyl-*S*-adenosyl methionine (1.54 Ci/mole); (c) protein synthesis mixture with T4 DNA, 12.95 nmoles of ¹⁴C-methyl-*S*-adenosyl methionine (3.08 Ci/mole). Enzyme reaction was allowed to proceed for 30 min at 30 C. Samples of 5 µliters were used for the chromatography. The thin-layer chromatographs were scanned with a Radio Scanner (Berthold, Varian Aerograph).

tants of gene 1 are not able to synthesize these late proteins. Polyacrylamide gel patterns obtained with these mutants in the early phase after infection are similar to those of the wild type, except that the gene 1 product disappears and is replaced by protein fragments resulting from the amber mutation (8). However, the gel pattern is very different in the late phase of infection. Whereas the proteins produced by the gene 1 mutants are almost the same at all times after infection, the protein pattern changes after wild-type T7 infection (8). The gene 1 product apparently changes the specificity of the ribonucleic acid (RNA) synthesis. In the absence of gene 1

FIG. 2. Inhibition of synthesis of *S*-adenosyl methionine cleaving enzyme by inhibitors of protein synthesis. Protein synthesis in the presence of added T3 DNA was performed as described in Materials and Methods. To the reaction mixtures were added: (a) no addition, $\square$ — $\square$ (b) fusidic acid, 10^{-8} M, $\triangle$; (c) chlortetracycline, 100 μ g/ml, $\square$ -- $\square$; (d) neomycin B, 200 μ g/ml, $\circ$. The enzyme was assayed in a total volume of 22 μ liters, which consisted of 2 μ liters of synthesized enzyme and 25.3 nmoles of 14 C-methyl-*S*-adenosyl methionine (0.527 Ci/mole). After incubation at 30°C for 30 and 60 min, 5- μ liter samples were removed, and the remaining *S*-adenosyl methionine and the split product were determined by thin-layer chromatography.

product, *E. coli* RNA polymerase can transcribe only the early regions of the T7 genome (9).

Our system for in vitro synthesis of enzymes requires transcription of the DNA that is added to the mixture. Since this system is derived from uninfected cells, the *E. coli* RNA polymerase present should transcribe only the genes for early proteins. Therefore, the ability of this DNA-dependent cell-free system to synthesize T7 lysozyme suggests that this enzyme is one of the two unknown proteins of the early infection phase. The conditions for T7 DNA-directed synthesis of lysozyme in vitro are similar to the conditions required for the synthesis of "early" proteins in response to DNA from T4 and *Bacillus subtilis* phage SP82 (5).

The T7 lysozyme made in vivo after phage infection has not been characterized yet. However, by the Sephadex gel filtration technique, we found that the molecular weight of the in vitro-synthesized T7 lysozyme seems to be about 17,000

FIG. 3. Kinetics of reaction of the in vitro-synthesized *S*-adenosyl methionine cleaving enzyme. A sample (50 μ liters) was incubated for protein synthesis with T3 DNA. A 2- μ liter portion was used for the enzyme reaction. In addition to the in vitro-synthesized enzyme, the reaction mixture contained 29.5 nmoles of 14 C-methyl-*S*-adenosyl methionine (6.77 Ci/mole) in a total volume of 40 μ liters. Samples of 5 μ liters were removed at the given times and analyzed by thin-layer chromatography for *S*-adenosyl methionine and for the split product.

(unpublished results). This would be similar to the molecular weights of the well-studied lysozymes from T4 [19,000 daltons (10)] and egg white [15,000 daltons (1)].

The phage T3 is closely related to T7 (7). One of the very few differences between them is the capacity of T3 to cause the induction of an enzyme which splits *S*-adenosyl methionine into thiomethyl adenosine and homoserine (2). The purpose of the induction of this enzyme by the phage could be the impairment of host functions, thereby favoring viral development. To serve this purpose in blocking some cell functions, one might expect that this enzyme should be synthesized early after phage infection. In vivo studies on the kinetics of the appearance of this enzyme agree with this expectation (2). Our results with T3 DNA-directed synthesis in vitro with a system from uninfected *E. coli* show again that this enzyme is synthesized; it also belongs to the class of T3 early enzymes. The synthesis in vitro of functioning gene 1 product which would switch on late functions is not required for the synthesis of lysozyme or of the *S*-adenosyl methionine cleaving enzyme: *E. coli* RNA polymerase transcribes the lysozyme gene in the absence of protein synthesis (4). Further, enzyme synthesis in vitro occurred also from DNA with an amber mutation

in gene 1 in a nonpermissive system (*unpublished results*, together with F. W. Studier).

The efficiency of the *in vitro* enzyme synthesis obtained in these experiments is remarkably high. Assuming that the specific enzymatic activities of the lysozymes of T7 and T3 are similar to that determined for T4, one can calculate that approximately 5 to 10% of the total leucine incorporated into protein is incorporated into active lysozyme. If the three early proteins are synthesized in equimolar amounts, and the molecular weights are those mentioned earlier, lysozyme, with a molecular weight of 17,000 daltons, should constitute 11% of the total protein synthesized. This means that a very high percentage of the *in vitro* amino acid incorporation goes into complete chains.

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