DIFFERENTIAL SYNTHESIS OF PROTEINS BY T4 PHAGE AND ITS HEAD PROTEIN AMBER MUTANT

S.G.Devare and J.D.Padayatty
Department of Biochemistry
Indian Institute of Science
Bangalore-12. India.

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SUMMARY

There was no difference in the incorporation of S-35 label into proteins of T4 and amber B17 phage grown on Escherichia coli B. The head protein peak was absent in the polyacrylamide gel electrophoretic profile of the S-35 labeled proteins of amber B17 grown on non-permissive host, E.coli B. However, an increase of 15-70% in the synthesis of other phage proteins of amber B17 over that of T4 phage was observed. The lysozyme activity increased by two fold in amber B17 in comparison with that of T4 phage grown on E.coli B. These results imply that in the absence of head protein synthesis by amber mutant there was an increase in the synthesis of other phage proteins.

Amber mutations cause premature termination of protein chains (1). The head protein of T4 phage is the major protein synthesized during the late period after infection (2). Amber mutation in the head protein gene may result in an increase in synthesis of other phage proteins when grown on a non-permissive host. This possibility was investigated by studying the synthesis of proteins of T4 phage and its head protein amber mutant, B17.

METHODS

Escherichia coli B cells grown in M9 medium (3) to 5×10^8 cells per ml, were harvested and infected with T4 or amber B17 phage at a multiplicity of five. They were incubated at 37° with shaking in M9 medium containing 50 μc of $^{35}SO_4^{2-}$ as the sole source of sulfur (purchased from Bhabha Atomic Research Centre, Bombay). Samples (0.1 ml) were withdrawn at

definite intervals of time and the proteins were precipitated with 10% (W/V) trichloroacetic acid. The precipitate was centrifuged, washed, dissolved in formic acid, plated on filter paper discs, dried and the radioactivity was measured in a Beckman LS-100 spectrometer.

In order to prepare ^{35}S labeled phage proteins, the T4 or amber B17 infected cells were allowed to grow upto 35 min. Cells were lysed with chloroform, cell debris removed by centrifugation, the proteins were precipitated and washed with trichloroacetic acid. The precipitated proteins were dissociated by heating in a boiling water bath for 2 min in 0.1 M phosphate buffer, pH 7.0, containing 2% sodium dodecyl sulfate and 2% mercaptoethanol (4). Equal amounts of the protein (T4 and amber B17 as determined by radioactivity) were applied on 7.5% acrylamide gels and subjected to electrophoresis in 0.1 M phosphate buffer, pH 7.0, containing 0.1% sodium dodecyl sulfate for 3 hr (4).

E.coli B cells grown in M9 medium containing 0.25% casamino acids to 5 x 10^8 cells per ml were harvested, infected with T4 or amber B17 at a multiplicity of five, and grown for 25 or 35 min. The cells were harvested, suspended in cold 0.05 M Tris-HCl buffer pH 7.5 and sonicated by an ultrasonic disintegrator for 2 min. The lysozyme activities were assayed using E.coli B cells sensitized with chloroform (5).

RESULTS AND DISCUSSION

The incorporation of ^{35}S label into proteins of T4 and amber B17 phage grown on E.coli B is shown in Table I. There was not much difference in the incorporation of the label into proteins of amber B17 and T4 grown on E.coli B. The

Table I. Incorporation of ^{35}S into proteins of T4 and amber B17 phage grown on E.coli B

Time after infection (min)	CPM T4	CPM B17
12	5,610	5,370
15	16,800	15,540
20	31,970	35,260
25	50,600	55,270
30	71,890	81,630

E.coli B cells grown in M9 medium (10 ml) to 5 x 10^8 cells per ml were harvested by centrifugation, suspended in M9 medium (1ml) and infected with T4 or amber B17 at a multiplicity of five. They were incubated in M9 medium (10 ml) containing 50 µc of S-35 sulfate as the sole source of sulfur at 37° with shaking. Samples (0.1 ml) were withdrawn at definite intervals of time and the proteins were precipitated with 10% trichloroacetic acid, using 0.1 ml of 0.2% bovine serum albumin as the carrier. The pricipitate was centrifuged, washed thrice with 5% cold trichloroacetic acid and once with ether. It was dissolved in 0.3 ml formic acid (90%) of which 0.2 ml was plated on filter paper disc, dried and counted in a Beckman LS-100 spectrometer using 0.5% 2,5 diphenyloxazole in toluene.

N-terminal fragment of the head protein synthesized by amber B17 grown on non-permissive host E.coli B does not contain any sulfur amino acid (1) and thus does not contribute to the incorporation of radioactivity. The proteins synthesized by T4 and amber B17 grown on E.coli B were separated according to their molecular weights by sodium dodecyl sulfate acrylamide gel electrophoresis (Figure 1). As expected the major head protein peak A, was negligible in the electrophoretic pattern of amber B17 proteins. However, there was an increase in the synthesis of other proteins (15-70%) as shown by the increase

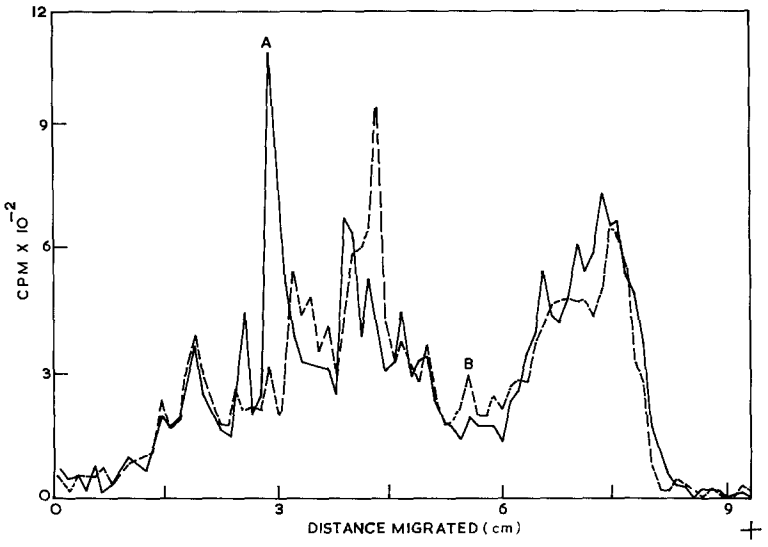

Figure 1. Polyacrylamide gel electrophoresis profiles of S-35 labeled proteins of T4 and amber B17 phage grown on E.coli B. E.coli B cells (5 x 10^9 cells in 1 ml) were infected with T4 or amber B17 at a multiplicity of five and grown in M9 medium (10 ml) containing 50 µc S-35 sulfate as the sole source of sulfur at 37° with shaking for 35 min. Cells were lysed with chloroform and the cell debris was removed by centrifugation (5000 x g). The proteins in the supernatant fraction were precipitated by cold trichloroacetic acid (10%), sedimented and washed with 5% cold trichloroacetic acid and ether. The precipitate suspended in 0.5 ml of dissociating buffer, 0.01 M phosphate buffer, pH 7.0 containing 2% sodium dodecyl sulfate (SDS) and 2% mercaptoethanol, was heated in a boiling water bath for 2 min. An aliquot 40,000 cpm (counted at an efficiency of 53%) was applied on 7.5% acrylamide gel (9.5 x 0.6 cm) and subjected to electrophoresis (8 mA per tube) for 3 hr in 0.1 M phosphate buffer pH 7.0 containing 0.1% SDS, (4). Bovine serum albumin, trypsinogen, pepsinogen, egg white lypozyme and cytochrome C (purchased from Sigma Chemical Co, Missouri, St.Louis) (10 µg each) were used as internal standards for the determination of molecular weights. These were stained by amido schwarz and the labeled phage proteins were identified by counting 1 mm slices of the gel on filter paper discs (6) (counted at an efficiency of 28%) in Beckman LS-100 scintillation spectrometer.___T4,--B17.

in peaks of radioactivity. The radioactivity from peak B of molecular weight, 18,000 daltons, showed an increase of about 45% in amber B17 protein, and may represent the structural component of the head (7), lysozyme (8) and core protein (9). This may imply an increase in the synthesis of lysozyme

protein in amber B17 over that of T4 grown on E.coli B. The lysozyme activities are shown in Table II. There was a two fold increase in the lysozyme activity in amber B17 over that of T4. Any effect due to lysis during growth of the amber B17 or T4 phage infected cells was avoided by using equal number of cells grown for 25 or 35 min for lysozyme assay.

These results show that there was an increase in the synthesis of other phage proteins when the head protein synthesis was prematurely terminated in the head protein amber mutant. The increase in translation coupled with or

Table II. Activities of lysozyme in T4 and amber B17 phage grown on E.coli B

Period (sec)	Optical density at 450 mu		
	Control	T4	B17
0	0.84	0.84	0.84
15	0.84	0.80	0.72
30	0.83	0.77	0.64
60	0.83	0.70	0.52
90	0.82	0.64	0.45
120	0.82	0.58	0.40
150	0.81	0.53	0.38

E.coli B cells were grown to 5 x 10^8 cells per ml in 100 ml M9 medium supplemented with 0.25% casamino acids. The cells were harvested, suspended in 1 ml of M9-casamino acid medium and infected with T4 or amber B17 at a multiplicity of five. They were incubated in 25 ml of M9-casamino acid medium for 25 min at 37° with shaking. The cells were harvested, equal number of infected cells were suspended in 2 ml of cold 0.05 M tris-HCl buffer, pH 7.5 and sonicated by ultrasonic disintegrator for 2 min in the cold. The lysate (0.1 ml) was added to 0.9 ml of E.coli B cells sensitized with chloroform and the decrease in optical density at 450 mμ at 25° was followed (5).

without transcription may result in the differential synthesis of T4 and amber B17 phage proteins.

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