INDUCTION OF A NEW ENZYME ACTIVITY TO EXCISE PYRIMIDINE DIMERS IN ESCHERICHIA COLI INFECTED WITH BACTERIOPHAGE T4

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SUMMARY: An extract of Escherichia coli infected with bacteriophage T4 contains an enzyme activity which releases specifically nucleotides containing pyrimidine dimers from ultraviolet-irradiated T4 DNA, pre-exposed to T4 endonuclease V. The enzyme activity increases after infection with T4 or $T4v_1$, and the increase is inhibited by chloramphenicol. The enzyme was partially purified and its properties were described.

Recent studies in this and other laboratories revealed that an endonuclease specific for ultraviolet (UV)-irradiated DNA is involved in repair of DNA. T4 endonuclease V, which induces single-stranded breaks in irradiated DNA in the absence of Mg^{++}, was isolated from Escherichia coli infected with bacteriophage T4 (1 - 3). The formation of the enzyme is controlled by the v^+ gene of T4, the gene which is known to increase survival of phage after UV irradiation (4). It was shown, moreover, that excision of dimers in vivo as well as in vitro take place in T4-infected cells but not in T4v-infected cells (1, 5). Thus, it seems probable that the enzyme is responsible for the first step of excision repair.

What enzyme(s) is involved in the second step of repair? This report describes the search for such an enzyme activity in T4-infected cells.

MATERIALS AND METHODS

E. coli N29, deficient in both DNA polymerase I and endonuclease I activities, was isolated from strain 1100 after mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine by Dr. K. Shimada and Mr. M. Katsuki. Bacteriophage T4 and its UV-sensitive mutant, $T4v_1$, were provided by Dr. W. Harm.

Preparation of [^{14}C]thymine-labeled T4 DNA and determination of pyrimidine dimers have been described (6). T4 endonuclease V, isolated from T4-infected E. coli 1100, was provided by Dr. S. Yasuda (2).

The excision enzyme was purified from an extract of T4v$_1$-infected E. coli N29. Cells were grown to 5 x 10^8 cells/ml at 37°C in broth and infected with T4v$_1$ at a multiplicity of 5. The infected cells were collected at 15 min after infection, suspended in 10 mM Tris·HCl-1 mM 2-mercaptoethanol (pH 7.5) at a concentration of 10^{11} cells/ml, and disrupted in an ultrasonic disintegrator. To 20 ml of the extract (30 mg protein/ml) were added with continuous stirring 2.32 g of 20 % (w/w) Dextran-500, 6.44 g of 30 % (w/w) polyethylene glycol-6000, and 6.66 g of NaCl. After stirring in the cold for 1 hr, the mixture was centrifuged at 1,000 x g for 20 min and the supernatant fluid was retained. Extraction of the pellet was repeated, and the combined supernatant was dialyzed against 10 mM Tris·HCl-1 mM 2-mercaptoethanol (pH 7.5). 11.5 g of ammonium sulfate was added gradually to the dialyzed fraction (70 ml) and the mixture, after stirring in the cold for 1 hr, was centrifuged at 1,000 x g for 15 min. 13.1 g of ammonium sulfate was added to the lower phase (61 ml), and the resulting precipitate was collected by centrifugation at 10,000 x g for 20 min and dissolved in 12.5 ml of 10 mM Tris·HCl-1 mM 2-mercaptoethanol-10 % ethylene glycol, pH 7.5 (TME buffer). After dialysis against TME buffer, the material (40 mg protein) was applied to a DEAE-cellulose column (1 x 13 cm). The column was eluted with a linear gradient of 0 - 0.3 M NaCl in TME buffer (200 ml). Fractions containing the activity, eluted at NaCl concentration of 0.05 M, were pooled and dialyzed against 10 mM potassium phosphate-1 mM 2-mercaptoethanol-10 % ethylene glycol (pH 6.5). Although further purification can be achieved by phosphocellulose chromatography, the DEAE fraction was used in most of these experiments.

RESULTS

Induction of an excision enzyme by infection with T4:

Table 1 summarizes the result of an experiment in which we compared the excision of pyrimidine dimers from irradiated T4 DNA by three types of extracts. The treatment of irradiated

Table 1. Release of thymine and its dimers from UV-irradiated T4 DNA by extracts of normal, T4-infected and $T4v_1$-infected cells

DNA	Extract	% release from DNA	
		Dimers	Thymine
Non-treated	None (0-time)	3.5	0.5
	Uninfected cells	2.3	0.6
	$T4v_1$-infected cells	2.9	0.9
	T4-infected cells	27.6	3.8
Treated	None (0-time)	2.4	0.4
	Uninfected cells	10.8	5.7
	$T4v_1$-infected cells	41.0	11.1
	T4-infected cells	43.0	10.4

[^{14}C]thymine-labeled T4 DNA was irradiated with UV (760 ergs/mm^2) and divided into two portions; one portion of DNA (50 µg/ml) was incubated with an extract of T4-infected E. coli 1100 (300 µg protein/ml) in 0.01 M EDTA-0.04 M Tris·HCl (pH 7.5) at 37°C for 60 min and then subjected to phenol extraction, while the other was left without treatment. Treated and untreated DNA samples were incubated at 37°C for 60 min in the following reaction mixture; 5 µg of DNA (2.3 x 10^4 c.p.m.), 5 µmoles of $MgCl_2$, 20 µmoles of Tris·HCl (pH 7.5) and 50 µg protein of extract in 0.5 ml. After incubation, the mixture was acidified by adding 0.05 ml of 6 N perchloric acid and separated into DNA and acid-soluble fraction. Each sample was hydrolyzed and radioactivity of thymine and its dimers was determined.

DNA with an extract of T4-infected cells results in the selective release of dimers, whereas the treatment with an extract of $T4v_1$-infected or non-infected cells induces no such release, in agreement with the previous results (1, 6). When, however, irradiated DNA which has been treated with a T4-infected cell extract in the presence of EDTA was used as the substrate, the selective release of dimers is induced by an extract of $T4v_1$-infected cells as well as by an extract of T4-infected cells. It seems, therefore, that the v^+ gene-controlled enzyme, T4 endonuclease V, and an excision enzyme are necessary for excision of dimers and that the $T4v_1$-infected

cell extract contains only the latter whereas the T4-infected cell extract contains both.

Only a small amount of dimer release is effected by an extract of normal E. coli even when irradiated DNA, pre-treated with the T4-infected cell extract, is used as the substrate. The dimer-excising activity increases rapidly after infection with T4 (Fig. 1). Moreover, the increase in enzyme activity is almost completely inhibited by chloramphenicol, added just prior to infection. Thus, it is evident that the excision enzyme is induced by infection with T4.

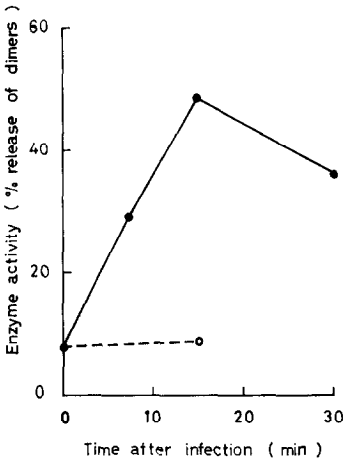

Fig. 1. Increase of an excision enzyme activity in $T4v_1$-infected cells. E. coli N29 (5×10^8 cells/ml) was infected with $T4v_1$ at a multiplicity of 5 and incubated at 37°C in broth. To one portion of the culture, chloramphenicol at 40 μg/ml was added 3.5 min prior to infection. At the times indicated, cells were collected and extracts were prepared. The excision enzyme activity was determined by using irradiated DNA, treated with T4-infected E. coli 1100, as the substrate. The procedures and conditions are as described in Table 1. ● —— ● $T4v_1$-infected cells, o --- o chloramphenicol-inhibited, $T4v_1$-infected cells.

Excision of dimers by purified enzymes: The excision enzyme was purified from $T4v_1$-infected E. coli N29 by phase partition in polyethylene glycol-Dextran followed by ammonium sulfate fractionation and DEAE-cellulose chromatography. The enzyme showed optimal activity at pH 7.0 in 10 mM $MgCl_2$-40 mM Tris·maleate buffer. The activity is dependent on the presence of Mg^{++}, and no release of dimers takes place in the presence of 10 mM EDTA.

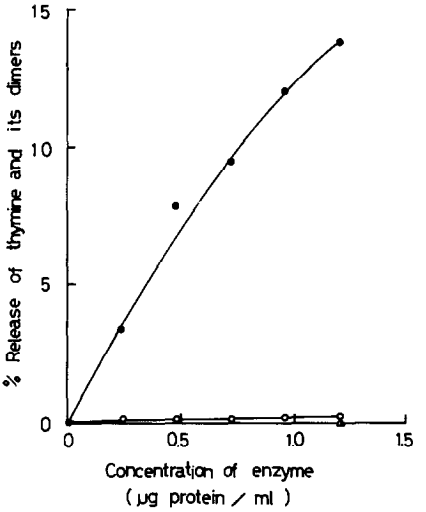

Fig. 2. Excision of pyrimidine dimers by purified preparations of T4 endonuclease V and excision enzyme. T4 DNA labeled with [^{14}C]thymine was irradiated at 760 ergs/mm^2. The pretreatment of DNA was performed at 37°C for 30 min in the following reaction mixture (2 ml); 50 μg of irradiated DNA, 3.1 μg of T4 endonuclease V (Hydroxylapatite fraction) and 80 μmoles of Tris·HCl (pH 7.5). After extraction with phenol and dialysis against 0.02 M NaCl, DNA was incubated with excision enzyme at 37°C for 30 min. The reaction mixture (0.5 ml) contains 2.5 μg of DNA (462 c.p.m. in dimers and 35,700 c.p.m. in thymine), 20 μmoles of Tris·maleate (pH 7.0), 5 μmoles of $MgCl_2$ and excision enzyme (DEAE fraction). Irradiated DNA, without endonuclease V treatment, was also incubated under the same conditions. Release from endonuclease V-treated DNA: ● —— ● dimers, o —— o thymine. Release from non-treated DNA: ▲ —— ▲ dimers, △ —— △ thymine.

The result shown in Fig. 2 indicates that pretreatment of irradiated DNA with T4 endonuclease V is essential for the specific release of dimers by the excision enzyme. The excision enzyme catalyzes little or no thymine or dimer release from irradiated DNA. When, however, irradiated DNA is pre-exposed to T4 endonuclease V before incubation with the excision enzyme, pyrimidine dimers are selectively released; 13.8 % of the dimers originally present in the DNA become acid-soluble, while only 0.26 % of the thymine is released from the DNA. From this result we can estimate that about 10 nucleotides are released for every dimer.

The preparation of excision enzyme does not seem to possess an endonuclease activity. In alkaline sucrose gradient

irradiated DNA, treated with the enzyme, sediments at almost the same rate as does non-treated DNA. It was shown, moreover, that little or no decrease in infectivity occurs when single- and double-stranded DNA of bacteriophage ØA are incubated with the enzyme (7).

DISCUSSION

It was suggested previously that excision of pyrimidine dimers from UV-irradiated DNA in T4-infected cells consists of two different steps (1). First, a single-stranded break is induced at a point close to a pyrimidine dimer, and second a nucleotide fragment containing dimer is released from the DNA; the former step does not require Mg^{++}, whereas the latter does.

Data presented in this and the previous papers clearly support this view. T4 endonuclease V, which induces single-stranded breaks specifically in UV-irradiated DNA in the absence of Mg^{++}, was isolated from T4-infected cells (2). The present experiments revealed that an extract of T4- or $T4v_1$-infected cells contains an enzyme activity that catalyzes the selective release of dimers in the presence of Mg^{++}. Since the enzyme possesses no endonuclease activity and pre-treatment of irradiated DNA with T4 endonuclease V is essential for excision of dimers by the enzyme, it is supposed that the excision enzyme acts at the nicks, produced by endonuclease V, and hydrolyzes a polynucleotide in a $5' \longrightarrow 3'$ direction to excise dimer-containing nucleotide from the DNA.

The dimer-excision activity increases rapidly after infection with T4 or $T4v_1$, and the increase is inhibited by chloramphenicol. Although several nucleases are known to be induced by T4 or T2, they differ from the excision enzyme in many respects (8 - 12). Thus, the enzyme may be a previously undiscovered nuclease induced by T4. A search for mutants unable to induce the enzyme activity is in progress.

Finally, it is pointed out that there is a low but significant dimer-excision activity in uninfected cell extracts. In these experiments we used a polA host mutant to reduce the level of excision activity in uninfected cells, since DNA polymerase I contains a dimer-excising nuclease activity (13). This implies that E. coli possesses a dimer-excising enzyme(s) besides DNA polymerase I.

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