A NEW DEOXYRIBONUCLEASE ACTIVITY FROM BACTERIA INFECTED WITH T5 BACTERIOPHAGE

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1. Introduction

We present evidence for the existence of a phage T5 induced enzyme which converts viral DNA molecules to high molecular weight fragments without the formation of acid-soluble products.

2. Materials and methods

Nuclease activity was routinely measured with the λDNA infectivity assay [1], using the clear plaque mutant λcb_2b_5 as a source of infectious DNA [2]. *Escherichia coli* Y10 (λ) was the source of wild type helper virus, and this strain was also used for the final plating in the assay. Competent bacteria were prepared by infection of *E. coli* Y10 (λ) with wild type λ. Anti-T5 rabbit serum was added to all plaque assay plates to inactivate any T5 phage present. The conversion of ^{32}P-labelled viral DNA to acid-soluble products was measured according to Paul and Lehman [3]. ^{32}P or ^{3}H labelled phage DNA preparations of high molecular weight were obtained by methods summarized by Thomas and Abelson [4]. Protein was measured according to Lowry et al. [5]. DNA from incubation mixtures was analysed by zone sedimentation through 5 ml density gradients of 5–20% (w/v) sucrose in 1 M NaCl - 0.01 M tris - Cl pH 7.2. The gradients were centrifuged at 0° in the SW 39 rotor of the Spinco Model L centrifuge. From each gradient, fractions of three drops were collected directly into scintillation vials from a hole punched in the bottom of the tube. Eight ml of scintillation fluid (7 g butyl PBD, CIBA, Duxford, Cambs; 80 g naphthalene; 600 ml toluene, 400 ml 2-methoxyethanol per l) was added to each vial, and the radioactivity measured in a Beckman scintillation counter using the isosets for ^{3}H and ^{32}P with ^{3}H. The counts were corrected for background and the overlap between the two channels.

3. Results

The T5 phage induced nuclease preparation was obtained as follows: a culture of the endonuclease-I deficient strain *E. coli* K12.1100 [6] was grown in broth to a cell density of 5×10^8/ml and infected with T5 phage. Five minutes later chloramphenicol was added to give a final concentration of 100 μg/ml, and the culture was poured on to crushed frozen 0.85% saline. The infected bacteria were collected by centrifuging, and resuspended at 2×10^{10} cells/ml in 0.02 M tris-Cl buffer pH 8.0 containing 5 M NaCl and 0.05 M $MgCl_2$ [7]. The bacteria were broken by treatment for 3 min in an MSE ultra-sonic desintegrator. Dextran T 500 (Pharmacia; 40 mg/ml of extract) and polyethylene glycol 600 (BDH; 60 mg/ml of extract) were added as dry solids and dissolved by stirring at 0° [7]. The suspension was centrifuged at 1000 × g for 20 min to separate the two phases; the top layer was removed and dialysed overnight at 4° against 0.01 M tris-Cl pH 7.2. A one ml portion of dialysed extract which contained a total of 100 μg of protein was mixed with one ml of a slurry of DEAE cellulose (0.27 mg/ml of Whatman DE-52 equilibrated with 0.01 M tris-Cl pH 7.2), and the suspension kept at 0° with occasional shaking. After 30 min the suspension was centrifuged and the DE-52 resuspended in 2 ml of 0.01 M tris-Cl pH 7.2

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Table 1
Summary of T5 phage deoxyribonuclease fractionation.

Fraction	Volume (ml)	Total protein (mg)	Percent of ^{32}P-DNA made acid-soluble by 0.1 ml of enzyme solution	Measured with λDNA infectivity assay		
				Total activity (units)	Specific activity (units/mg protein)	Purification (fold)
Crude extract	6.0	4.2	54	780	186	-
Top layer of phase separation	4.5	0.63	46	2700	4290	23
DE-52 eluate	5.0	0.55	14	2250	4080	22
Calcium phosphate gel eluate	2.5	0.045	0	1615	35900	193

The amounts of ^{32}P-labelled DNA made acid-soluble were measured after incubating 0.1 ml of enzyme solution with 0.1 ml of ^{32}P-labelled T5 phage DNA (4 · 8 cpm/μg) at 37° for 30 min. Very similar amounts of acid-soluble material were obtained when the same T5 DNA sample was first heat-denatured. One unit is the amount of enzyme which inactivates 10^9 molecules of λDNA per hour as measured in the infectivity assay.

containing 0.1 M NaCl. The DE-52 was eluted for 30 min at 0° and then separated by centrifuging. The elution was repeated with 2 ml of buffer containing 0.25 M NaCl. After dialysis against 0.01 M tris-Cl pH 7.2, the tris-0.25 M NaCl eluate was mixed with 0.5 ml of 0.2 M calcium phosphate gel [8], and kept at 0° for 45 min. After centrifuging, the supernatant was removed and the gel gently resuspended in 2 ml of 0.1 M phosphate buffer pH 7.4. After 45 min at 0°, the suspension was centrifuged, the supernatant removed, and the same procedures used to elute the gel with successive 2 ml batches of 0.2 M, 0.3 M, 0.4 M, 0.5 M phosphate buffer, and finally 0.5 M phosphate buffer containing 0.05 M citrate to dissolve the gel and to remove all protein. The phage T5 induced nuclease activity was found in the 0.4 M phosphate eluate.

The whole of the above procedure was applied simultaneously to an ultra-sonicated extract of uninfected bacteria. All fractions from infected and uninfected cultures were routinely dialysed against 0.01 M tris-Cl pH 7.2, and tested by the λDNA infectivity assay, and for their ability to convert native and heat-denatured ^{32}P-labelled T2 and T5 DNA to acid-soluble fragments. Although both of the crude extracts, and both of the top fractions from the phase separation readily solubilized ^{32}P-DNA and rapidly inactivated λDNA, the extract from T5 infected bacteria showed activity about 2–3 fold greater than that from uninfected bacteria in the latter assay. Activity detectable by both types of assay was also found in each of the 0.25 M NaCl eluates from DE-52. However, most of the contaminating nucleases which are detectable by the ^{32}P-DNA assay were removed from DE-52 by tris-0.1 M NaCl. The remainder of this activity was removed from both extracts by the calcium phosphate gel, from which it was eluted by 0.2 M phosphate buffer. The activity detectable only with the λDNA infectivity assay was found in the 0.4 M phosphate gel eluate from the infected cell extract, but not in any of the gel fractions from the uninfected culture. The enzyme purification is summarized in table 1. The apparent gain in total activity in the phase separation is unexplained. It could be caused by an inhibition of the enzyme's action or by inhibition of the λDNA infectivity assay by substances present in the crude extract, and which are removed in the phase separation.

The properties of the T5 induced nuclease were further investigated by using zone sedimentation to analyse the products formed from λDNA. The incubation mixture contained 0.1 ml enzyme (65 units) in 0.01 M tris-Cl pH 7.2, 0.02 ml of 0.2 M

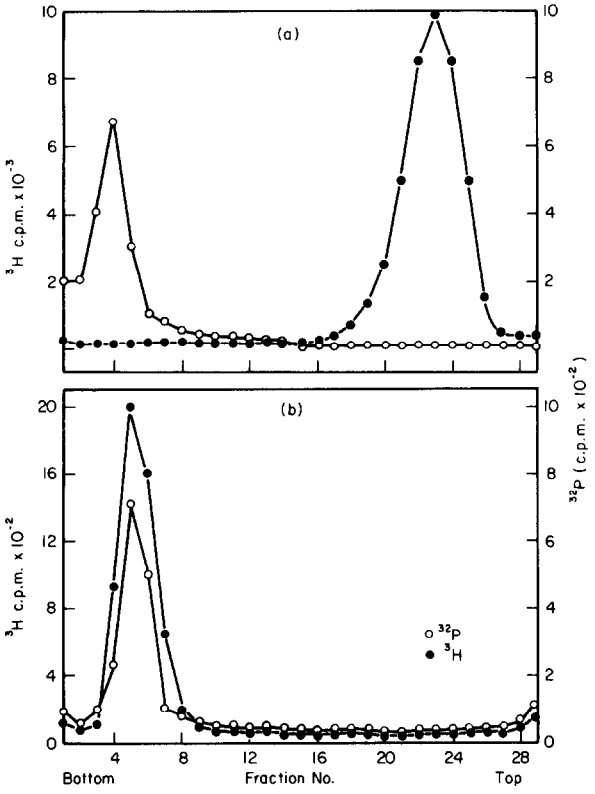

Fig. 1. Zone sedimentation analysis of products formed by T5 nuclease on ^{3}H labelled phage λDNA, with ^{32}P-λDNA, as a sedimentation marker. •——• ^{3}H counts; o——o ^{32}P counts. (a) Result using gel fraction from infected culture, (b) result using corresponding fraction from uninfected culture. Assuming a molecular weight of 31 X 10^{6} for λDNA, the midpoint of the ^{3}H peak in (a) corresponds to a molecular weight of about 5 X 10^{5} [10]. The sedimentation patterns was as that obtained in (a) when the enzyme incubation continued for 50 min.

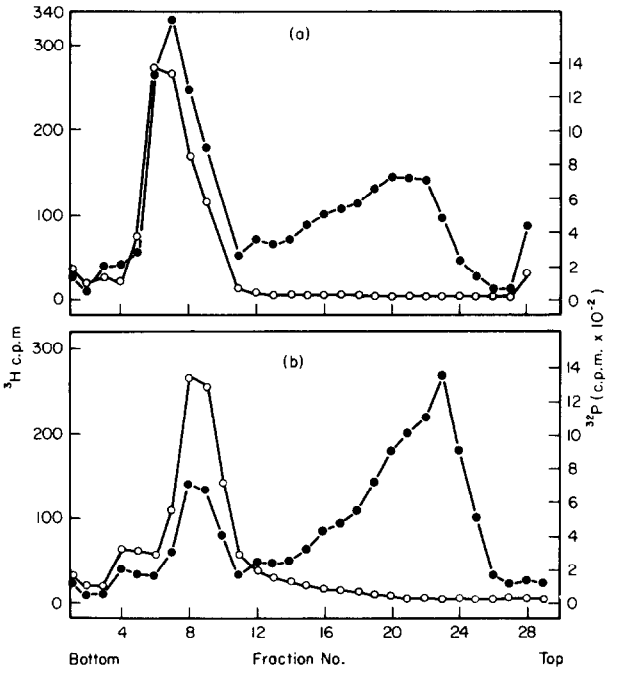

Fig. 2. Zone sedimentation of the products formed by T5 nuclease on ^{3}H-phage T5 DNA. Sedimentation was for 3 hr at 35000 rpm with ^{32}P-T5 DNA as a marker. (a) 10 Min incubation, (b) 40 min incubation. •——• ^{3}H counts; o——o ^{32}P counts. The distances sedimented by the marker DNA and the reaction products indicate that the latter have molecular weights over the range 1.2–27 X 10^{6}, with the peak region in (b) corresponding to a molecular weight of 4.4 X 10^{6} [10, 11].

$MgCl_2$ and 39 μg of ^{3}H labelled phage λDNA of specific activity 9.2 X 10^{3} cpm/μg. The control contained 0.1 ml of the corresponding gel eluate from the uninfected cells' extract. After 15 min at 37° the mixtures were chilled and deproteinized by rocking them gently with phenol. Samples from each mixture were layered on to the top of separate 5 ml sucrose density gradients together with unincubated ^{32}P-λDNA as a sedimentation marker. The gradients were centrifuged for 5 hr at 35,000 rpm and the radioactivity in the drop fractions measured as described in the Methods section. The result is shown in fig. 1. Exactly similar procedures were used to investigate the action of the same enzyme fraction on T2 and T5 viral DNAs. The result of the experiment with T5 DNA is shown in fig. 2. The result of the experiment with T2 DNA was similar to that with T5 in that conversion of the substrate DNA to fragments with a large range in molecular weight (6–60 X 10^{6}) occurred, and was incomplete after a 10 min incubation period.

We find that Mg^{++} ions are essential for the enzyme activity. No other cofactor requirement has been detected.

4. Discussion

The role of the enzyme in T5 infected bacteria is not clear. It is detectable at the time when the rapid degradation of the host cell DNA is initiated [9] and at a time when the infected bacteria first develop the ability to degrade the DNA of superinfecting heterologous phage, such as T2, T4 or λ (Fielding and Lunt, unpublished observations). However, the enzyme's behaviour *in vitro* is not compatible with sufficiently high specificity to distinguish between T5 and other DNA molecules. The large sizes of the fragments produced by the enzyme suggest that it might be recognizing specific nucleotide sequences in the DNA substrate molecules. This possibility can only be tested by detailed examination of the enzyme's mode of action.

Acknowledgement

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