JOURNAL OF VIROLOGY, Oct. 1981, p. 305-308
0022-538X/81/100305-04$02.00/0

Vol. 40, No. 1

Baculovirus Induction of a DNA Polymerase

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Received 22 April 1981/Accepted 15 June 1981

The baculovirus, *Autographa californica* nuclear polyhedrosis virus, induced a new aphidicolin-sensitive, α-like, DNA polymerase upon infection of the lepidopteran noctuid, *Trichoplusia ni*. The new virus-induced DNA polymerase could be separated from the host α-like polymerase by phosphocellulose chromatography. The two polymerases differed in their sensitivities to heat inactivation, high salt concentrations, and 0.1 M phosphate buffer.

The worldwide use of baculoviruses as biological pesticides has generated interest in the nature of this group of insect pathogens and the mechanism by which they infect their host (10, 11). An important aspect of understanding the mechanism of viral infections is determining the biochemical basis for selective amplification of the viral genome. Baculoviruses possess a large, circular, double-stranded DNA genome which replicates in the nuclei of infected host cells and is characteristically packaged in an enveloped, rod-shaped capsid (12). In this report we demonstrate the induction of a new, aphidicolin-sensitive DNA polymerase upon infection of a lepidopteran noctuid with the model baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV).

Comparison of DNA polymerase activities in post-mitochondrial supernatant fractions prepared from AcNPV-infected (10 PFU/cell) and mock-infected *Spodoptera frugiperda*-cultured cells (7, 8) revealed a sixfold increase in polymerase activity at 26 h postinfection when assayed in 50 mM potassium phosphate buffer (pH 7.2), 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 100 μM deoxyribonucleoside triphosphates, and 400 μg of activated calf-thymus DNA per ml (1). A similar observation of increased DNA polymerase activity upon infection of *S. frugiperda* cells with *Trichoplusia ni* nuclear polyhedrosis virus was recently reported (5). To obtain sufficient quantities of polymerases for further analysis of this phenomenon, third instar larvae of the cabbage looper, *T. ni*, were deprived of food overnight and then infected per os with the L-1 strain of AcNPV by introducing 10^8 occluded viruses (polyhedra) per cm^3 of food (8). Uninfected control larvae were concurrently maintained. Larvae were collected at 4 days postinfection and frozen at −70°C. Moribund larvae of the infected group were collected for further enzyme work. Post-mitochondrial supernatant fractions were prepared and fractionated by chromatography on phosphocellulose columns (Fig. 1). The buffers contained 1 mM phenylmethylsulfonyl fluoride and 10 mM sodium bisulfite to minimize proteolysis (2) which was problematic in *Drosophila melanogaster* DNA polymerase isolation (3).

When the post-mitochondrial supernatant fraction from uninfected *T. ni* larvae was applied to the phosphocellulose column, a single peak of polymerase activity eluted with 0.25 M KCl (Fig. 1A). In contrast, two peaks of polymerase activity eluted with 0.25 M KCl and 0.4 M KCl when a post-mitochondrial supernatant fraction from infected *T. ni* larvae was applied to phosphocellulose column (Fig. 1B). The polymerase eluting at 0.25 M KCl will be referred to as the host-cell DNA polymerase, and the polymerase eluting at 0.4 M KCl will be referred to as the AcNPV-induced DNA polymerase.

Since only one of the three types of eucaryotic DNA polymerases, the α-polymerases, exhibit sensitivity to the diterpenoid inhibitor aphidicolin (6, 13), it was of interest to determine the sensitivity of the two DNA polymerases from infected *T. ni* larvae to aphidicolin inhibition. The results (Fig. 2) demonstrate that both the host cell and AcNPV-induced DNA polymerases have similar, if not identical, sensitivities to aphidicolin. Approximately 50% inhibition of activity of both polymerases is achieved with 0.25 μg of aphidicolin per ml. The data indicate that both enzymes are α-like DNA polymerases, the type generally associated with nuclear DNA replication (6). Although the presence of a β-like DNA polymerase in uninfected *T. ni* larvae was not extensively investigated, the apparent absence of a β-like enzyme has been noted in *D. melanogaster* and a variety of lower eucaryotes (2, 6).

The two α-like DNA polymerases have several distinguishing features which are summarized in

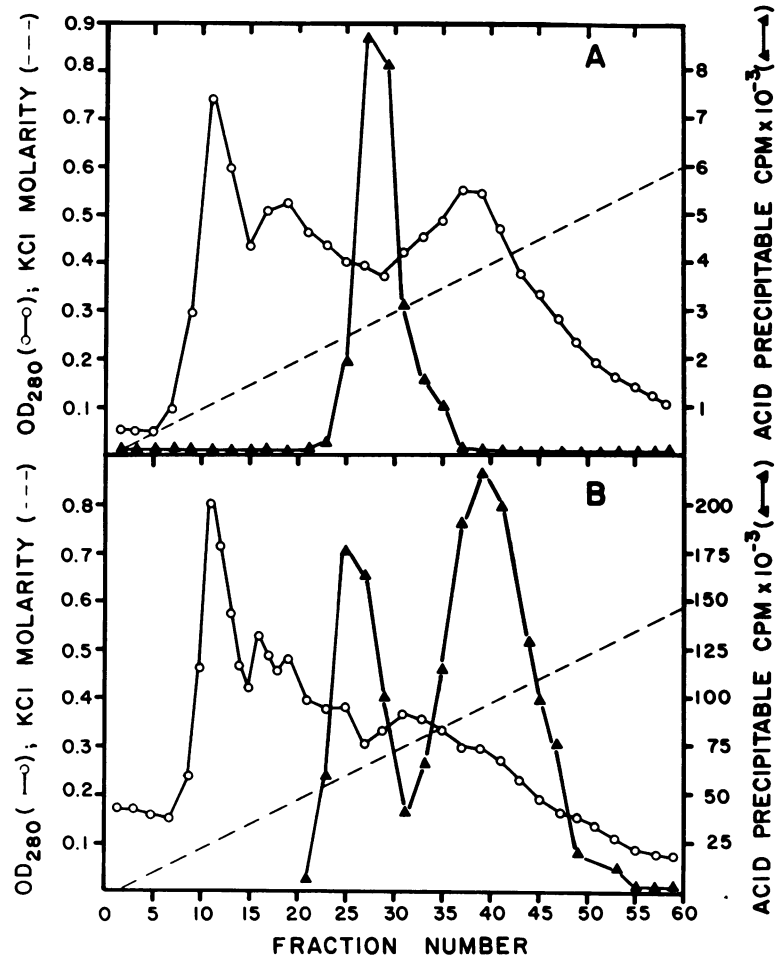

FIG. 1. *Phosphocellulose column profiles of DNA polymerase activity from uninfected and infected larval extracts. Approximately 10 g of frozen T. ni larvae were macerated in 25 ml of extraction buffer (EXB) containing 50 mM potassium phosphate (pH 7.2), 10 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium bisulfite, and 10% glycerol. The mixture was homogenized in a glass and Teflon Potter-Elvejhem homogenizer and centrifuged for 30 min at 82,500 × g at 5°C. The supernatant was applied directly to a phosphocellulose column (2.6 by 10 cm) equilibrated with EXB. After sample application, the column was washed with EXB until the optical density at 280 nm (OD_{280}) of the eluate declined to approximately 0.2. A 250-ml, linear 0 to 0.67 M KCl in EXB gradient was applied. Fractions, approximately 4.5 ml each, were collected and assayed for DNA polymerase activity. The assay was performed in 50 mM potassium phosphate (pH 7.2), 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 100 μM dATP, dGTP, and dTTP, 400 μg of activated calf thymus DNA per ml, and tracer quantities of [α-^{32}P]dCTP, usually 0.1 μCi per assay. The 50-μl assay mixture was incubated at 32°C, and a sample was withdrawn at 30 min and applied to a small semicircle of Whatman 3 MM filter paper which had been presoaked in 1 mM ATP and dried before use. The filter papers were immersed in ice-cold 5% trichloroacetic acid (approximately 10 ml per filter paper) and gently swirled for 15 min. The trichloroacetic acid washes were repeated three times, and the filters were then washed once with cold 95% ethanol, finally dried, and counted in a scintillation counter. (A) Uninfected larval extract; (B) AcNPV-infected larval extract.*

Table 1. The AcNPV-induced polymerase is much more sensitive to heat inactivation at 45°C than the host cell polymerase. Notably different effects on enzyme activity are observed with 0.25 M KCl and with 0.1 M phosphate; AcNPV-induced DNA polymerase is stimulated approximately 2.5-fold by these conditions, whereas the host cell polymerase is inhibited. The results collectively indicate that the viral-induced enzyme is different than the host cell polymerase. Thus, the viral-induced polymerase is probably a distinct, new polymerase activity although a

substantial viral modification of the preexisting host cell polymerase may also explain the different polymerase properties.

Direct confirmation that a new α-like polymerase is encoded fully of in part by the 8.7×10^7-dalton AcNPV DNA genome (9) must await correlation with viral genetic mutants (4, 8). On the strength of the data presented herein, it will be valuable to screen the growing collection of temperature-sensitive AcNPV mutants for reduced levels of viral-induced DNA polymerase activity at the restrictive temperature. The research opens many new avenues to explore concerning the process of baculovirus replication.

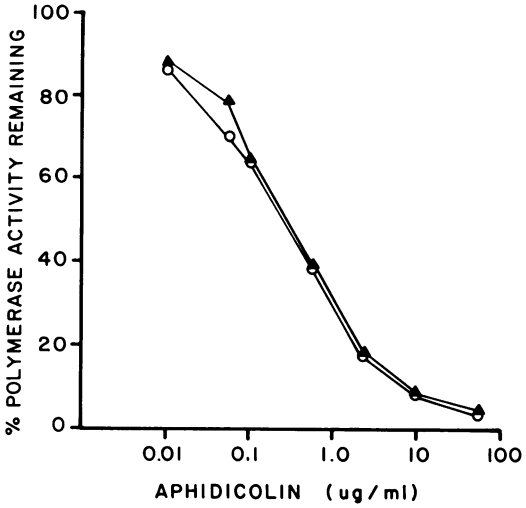

FIG. 2. *Effect of aphidicolin on host cell and AcNPV-induced DNA polymerase activity. Peak fractions from phosphocellulose columns (Fig. 1B) containing host cell and AcNPV-induced DNA polymerase activities were separately pooled. Solid ammonium sulfate was added to the AcNPV-induced DNA polymerase to 40% saturation. The resulting suspension was centrifuged at 16,000 × g for 30 min. To the supernatant, additional ammonium sulfate was added to 70% saturation and again centrifuged at 16,000 × g for 30 min. The pellet was resuspended in extraction buffer (see legend to Fig. 1). Assay of this 40 to 70% ammonium sulfate fraction demonstrated 85% recovery of the original polymerase activity. Both host cell and AcNPV-induced DNA polymerases were then dialyzed overnight against extraction buffer and stored in liquid nitrogen until further use. A 2-mg/ml solution of aphidicolin (gift of John Douros, National Cancer Institute, Bethesda, Md.) was prepared in dimethyl sulfoxide and diluted appropriately into assay mixtures containing 50 mM potassium phosphate (pH 7.2), 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 50 μM of all four deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, and dTTP), 1 mg of activated calf thymus DNA per ml, and 1 Ci of [α-^{32}P]dCTP per mmol. Control incubations with equivalent amounts of dimethyl sulfoxide were also tested. Samples were withdrawn after 30 min of incubation at 32°C, and trichloroacetic acid-precipitable counts were determined as described in the legend to Fig. 1. Results are expressed as percent of activity in the absence of aphidicolin. Host cell DNA polymerase (▲); AcNPV-induced DNA polymerase (○).*

TABLE 1. *Effects of various incubation conditions on host cell and AcNPV-induced DNA polymerases*

Incubation conditions	Activity of polymerase (%)	
	Host cell	AcNPV-induced
Standard rxn[a]	100	100
+0.1 M KCl	155	250
+0.25 M KCl	25	240
+0.1 M NaCl	75	150
+0.25 M NaCl	22	61
+0.1 M PO_4^{2-} (pH 7.2)	89	240
Heat-treated enzyme[b]	70	10

[a] Conditions used were the same as those in Fig. 2.
[b] Heat treatment of enzyme involved a 20-min incubation period at 45°C in extraction buffer.

This research was supported in part by the U.S. Public Health Service Grant 9 ROI AI17338-04 from the National Institute of Allergy and Infectious Diseases. J.E.J. was a participant in the Undergraduate Research Program at the University of Idaho sponsored by the National Science Foundation SMI-76-83285. This paper was published with the approval of the director of the Idaho Agricultural Experiment Station as research paper number 8152.

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