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Deoxyribonucleic Acid-Dependent Nucleotide Phosphohydrolase Activity in Purified Vaccinia Virus

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A nucleotide phosphohydrolase (adenosine triphosphatase), which is associated with vaccinia virus cores, has been solubilized and shown to be deoxyribonucleic acid dependent.

Vaccinia virions contain at least five enzymatic activities: these include a deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase (12, 14), a nucleotide phosphohydrolase (adenosine triphosphatase; references 8, 15), two deoxyribonucleases (3, 18), and a protein kinase (17). None of the enzymes has yet been extracted from vaccinia virus cores and purified. In this note, we describe the solubilization of the nucleotide phosphohydrolase and the unexpected finding that the enzymatic activity is dependent on DNA.

Although the nucleotide phosphohydrolase can be released from the virus particle with sodium deoxycholate (Paoletti and Munyon, *unpublished results*), little activity remains in the supernatant fraction after high-speed centrifugation (Table 1, minus DNA). Further experiments with ^{3}H-thymidine-labeled vaccinia virus indicated that the viral DNA is released during the deoxycholate treatment and that less than 10% is sedimented at 38,000 × *g* but greater than 95% is sedimented at 136,000 × *g*. This observation led us to consider that the nucleotide phosphohydrolase might be a DNA-dependent enzyme and that loss of activity after high-speed centrifugation results from sedimentation of the viral DNA. DNA was added to the virus extracts to test this hypothesis. Addition of denatured salmon sperm DNA to the 136,000 × *g* supernatant fraction leads to more than a 200-fold stimulation of adenosine triphosphatase activity (Table 1). When the 136,000 × *g* supernatant fraction is sedimented on a glycerol gradient, a single major peak of DNA-dependent nucleotide phosphohydrolase activity is detectable (Fig. 1). Similar results are obtained whether the nucleotide phosphohydrolase is extracted from whole virus or

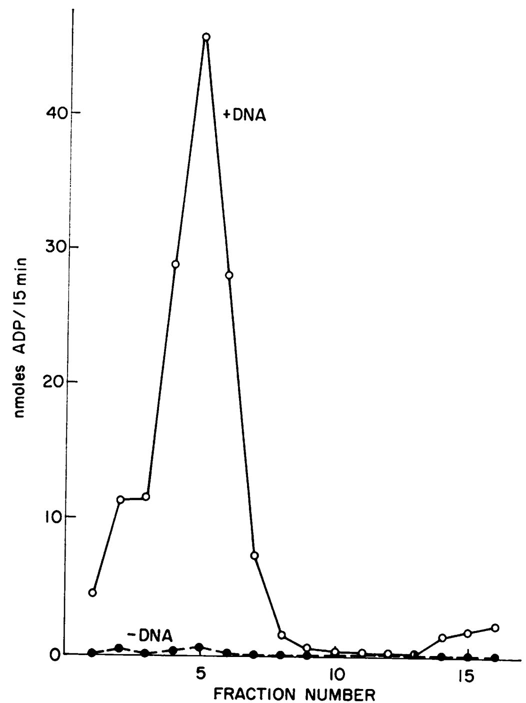

FIG. 1. *Glycerol gradient sedimentation of the vaccinia virus nucleotide phosphohydrolase. The 136,000 × g supernatant fraction was obtained after deoxycholate extraction of vaccinia virus as in Table 1. The concentration of virus in the dissociation mixture was optical density at 260 nm of 58 per ml. A 200-μliter portion was applied to a 10 to 30% glycerol gradient in 0.1 M Tris-hydrochloride, pH 8.0, 0.05 M dithiothreitol. After centrifugation in the SW65 rotor at 60,000 rev/min for 14.5 hr, 15-drop fractions were collected from the bottom of the tube. Nucleotide phosphohydrolase activity was measured as in Table 1.*

TABLE 1. *Stimulation of nucleotide phosphohydrolase activity by DNA*[a]

Virus fraction	Nanomoles of ADP/15 min		Fold stimulation
	−DNA	+DNA	
Untreated virus	14.9	76.2	5.1
Deoxycholate-treated virus			
Total	12.5	62.8	5.0
38,000 × *g* supernatant fraction	5.2	49.6	9.5
136,000 × *g* supernatant fraction	0.1	28.2	282.0

[a] Vaccinia virus, purified by sedimentation through a sucrose cushion and two sucrose gradient centrifugations (10, 13), at a concentration of optical density at 260 nm of 29 per ml was dissociated with 0.3 M tris(hydroxymethyl)aminomethane (Tris), *p*H 8.4, 0.05 M dithiothreitol, and 0.1% sodium deoxycholate at 0 C for 15 min. The mixture was centrifuged at 38,000 × *g* for 30 min at 4 C and the supernatant fraction was recovered. This supernatant fraction was then centrifuged at 136,000 × *g* for 1 hr. Equivalent amounts of untreated virus, total deoxycholate-treated virus, and low- and high-speed supernatant fractions were tested for adenosine triphosphatase activity. The reaction mixture (0.1 ml) contained 0.1 M Tris-hydrochloride, *p*H 8.4; 1 mM $MgCl_2$; 50 mM dithiothreitol; 1 mM ^{14}C-ATP, specific activity 200 counts per min per nmole; 0.1% Nonidet P-40 detergent; 25 μg of denatured salmon sperm DNA (Calbiochem) where indicated. After incubation at 37 C for 5 and 15 min, 10-μliter samples were spotted on top of dried nucleotide standards on PEI cellulose (J. T. Baker Chemical Co.). The chromatography plates were developed with 1 N CH_3COOH-4 M LiCl (8:2, v/v) as previously described (19). ADP spots observed under ultraviolet light were cut out, and radioactivity was measured in a liquid scintillation counter.

cores. Additional experiments indicate that native salmon sperm DNA is less effective than denatured DNA and that yeast transfer RNA is entirely ineffective. The fivefold stimulation of nucleotide phosphohydrolase activity in "untreated virus" (Table 1) presumably results from the partial disruption of the virion by Nonidet P-40 detergent and dithiothreitol, which are present in the reaction mixture.

DNA-dependent enzymes, which hydrolyze adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and P_i, are found in uninfected and phage-infected bacteria (1, 2, 5–7, 9, 16, 20, 22, 23) but have not previously been reported in animal cells or viruses. Certain of the bacterial enzymes also have ATP-dependent deoxyribonuclease activity and are thought to function in recombination, whereas others are without detectable deoxyribonuclease activity. It will not be possible to determine the presence of a coupled ATP-dependent deoxyribonuclease activity until the vaccinia virus nucleotide phosphohydrolase is obtained in a highly purified form, since both the endo- and exonuclease activities are released from vaccinia virus by the deoxycholate treatment (Rosemond, Paoletti, and Moss, *unpublished results*).

Reovirus (4, 11) and frog virus (21) also contain nucleotide phosphohydrolase activities within their cores. We suggest that tests be made for RNA and DNA dependence during attempts to purify these enzymes.

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