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Inhibition of DNA Polymerase from Herpes Simplex Virus-Infected Wi-38 Cells by Phosphonoacetic Acid

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Infection of Wi-38 cells with herpes simplex virus induced an elevated DNA polymerase activity which had many biochemical properties different from normal cell DNA polymerase. Phosphonoacetic acid specifically inhibited the virus-induced DNA polymerase as compared to the normal WI-38 cell DNA polymerase. The compound did not appear to inhibit enzyme activity by interacting with the DNA primer.

We have previously shown that phosphonoacetic acid inhibited herpes simplex virus (HSV) replication in model animal systems (8) and in tissue culture (6). The latter studies indicated inhibition of a late event in virus replication, and a possible suppression of viral DNA synthesis. However, it was uncertain whether the observed suppression was due to a direct inhibition of DNA polymerase or an indirect inhibition through interference with other steps of virus production. Polymerase activity in lysed cultures of infected cells was inhibited strongly by phosphonoacetic acid while the enzyme from normal cells appeared to be resistant. To confirm this differential sensitivity to phosphonoacetic acid the polymerases from both cultures were extensively purified and studied.

Methods for the growth of Wi-38 cell and HSV, type 1, have been described previously (6). Normal Wi-38 DNA polymerases were prepared from 56 roller bottles of cells harvested at 75% confluency. HSV-induced DNA polymerase was prepared from 42 roller bottles of confluent Wi-38 cells infected with HSV at a multiplicity of 10 mean tissue culture infective dose units per cell. The cultures were harvested when 20 to 50% of the cells showed typical cytopathic effect of HSV.

The cells were lysed and the polymerases from both cultures were purified by three column chromatographic steps (DEAE-cellulose, phosphocellulose, and Sephadex G-200) according to the procedure described by Smith and Gallo (9). The DEAE-cellulose columns gave DNA polymerase eluting as broad peaks centering around 0.2 M NaCl from HSV-infected cells and around 0.25 M NaCl from normal Wi-38 cells. The phosphocellulose column yielded a single DNA polymerase peak from HSV-infected cells, eluting at about 0.25 M KCl. Normal cells gave evidences of containing the expected three DNA polymerases (5). A major activity eluted at about 0.3 M KCl with a shoulder, and a minor activity eluted at 0.4 M KCl. All host DNA polymerases were eluted at higher KCl concentration than HSV DNA polymerase (Fig. 1). Further purification of each of these DNA polymerase activities was carried out on Sephadex G-200 columns (9). The standard reaction mixture (300 μl) for Wi-38 cell DNA polymerase contained 0.2 mmol each of unlabeled dCTP, dGTP, and dTTP, 0.5 μCi of dATP (specific activity 0.8 Ci/mmol), 50 μg of activated calf thymus DNA (1), 50 mM Tris-hydrochloride buffer (pH 7.8), 8 mM $MgCl_2$, and 1 mM dithiothreitol. The standard reaction mixture for HSV DNA polymerase was identical but contained in addition 150 mM KCl. Reaction times were 30 min for HSV DNA polymerase and 1 h for Wi-38 polymerase at 37 C. The amount of enzyme was selected to give a linear rate of incorportation for 1 h. The reaction was stopped by addition of 3 ml of cold 5% trichloroacetic-0.01 M sodium pyrophosphate. The acid-insoluble material was collected and washed on a membrane filter and counted in a scintillation spectrometer.

HSV-induced DNA polymerase was shown to be different from normal Wi-38 cell polymerases by several criteria. (i) The HSV polymerase was eluted at a lower salt concentration than the host polymerases on DEAE-cellulose and phosphocellulose; (ii) KCl stimulated HSV polymerase but inhibited host polymerase; (iii) The optimal Mg^{2+} concentrations were different. Similar results have been reported by other laboratories (2, 3, 11). In addition, we found several other differences between the normal

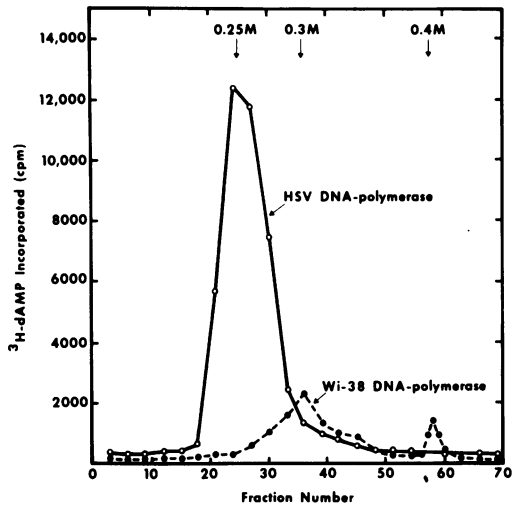

FIG. 1. *Phosphocellulose column chromatography of DNA polymerases. The active fractions from each DEAE-cellulose column were pooled and diluted to 0.1 M NaCl with DET buffer (1 mM dithiothreitol, 1 mM EDTA, 50 mM Tris-hydrochloride, pH 7.8) containing 20% glycerol. The samples were then passed through separate phosphocellulose columns (1.5 by 24 cm) which had been equilibrated with DET buffer containing 20% glycerol and 0.1 M KCl. After a wash with 50 ml of the equilibration buffer, each column was eluted with 560 ml of KCl linear gradient from 0.1 to 0.7 M in 20% glycerol-DET buffer. The flow rate was 0.4 ml/min and 5-ml fractions were collected. Fifty microliters of each fraction were used for DNA polymerase assay as described in the text.*

host polymerases and HSV-induced polymerase. First, 0.2 mM MnCl completely inhibited HSV polymerase but the host polymerases were only inhibited by 35%. Second, the HSV polymerase was more sensitive to inactivation by *N*-ethylmaleimide than the host polymerases. Third, the saturation concentration of activated calf thymus DNA as primer was 3 μg/ml for HSV polymerase but was 50 μg/ml for Wi-38 polymerases.

The effect of phosphonoacetic acid on the partially purified DNA polymerases from HSV-infected and uninfected Wi-38 cells is shown in Fig. 2. HSV enzyme was extremely sensitive to this compound with 50% inhibition at 0.2 μg/ml. In contrast Wi-38 enzymes, both the major activity which was a mixture of two host DNA polymerases and the minor activity, separated by phosphocellulose, were relatively resistant to this compound. The highest concentration of this compound tested against the host enzymes was 82 μg/ml which caused only a 10 to 15% inhibition.

There are many antibiotics and synthetic compounds, such as actinomycin (7), oligomycin, daunomycin, chromomycin (10), acridine (4), and ethidium bromide (10) that inhibit DNA synthesis. However, these inhibitors interfere with DNA synthesis by forming a complex with template DNA. This type of action usually lacks specificity and the compounds inhibit all DNA-primed enzymes. Phosphonoacetic acid apparently did not interact with template DNA, since the same template was used for HSV and Wi-38 DNA polymerases; yet, the drug inhibited only the former enzyme. Inhibition could not be overcome by increased amounts of primer DNA as shown in Table 1. The saturation concentration of DNA for HSV

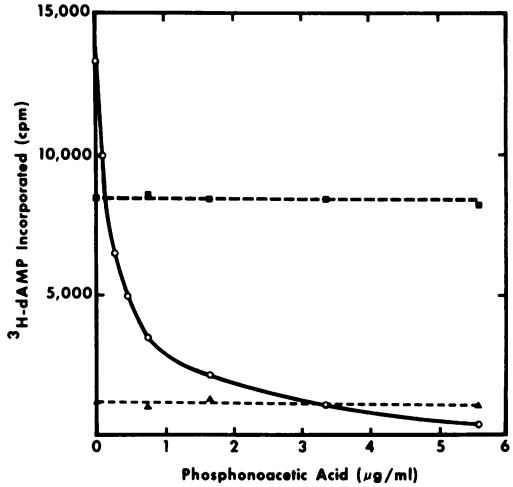

FIG. 2. *Effect of phosphonoacetic acid on the activity of Wi-38 and HSV DNA polymerases. HSV DNA polymerase* (○), *the major DNA polymerases of Wi-38 cells* (■), *and the minor polymerase of Wi-38 cells* (▲) *from phosphocellulose columns were tested against phosphonoacetic acid. Standard assays were as described in the text.*

TABLE 1. *Inhibition of HSV-induced DNA polymerase by phosphonoacetic acid (PAA) (0.3 μg/ml) at various concentrations of template DNA*

DNA concentration (μg/ml)	[^{3}H]AMP incorporated		% Inhibition
	− PAA	+ PAA	
0	153	142	
0.6	6,650	2,058	69
1.5	10,925	3,149	71
3	13,677	3,966	71
10	13,828	4,276	69
50	13,317	3,985	70
300	12,312	3,800	69

polymerase was 3 μg/ml as mentioned before. Phosphonoacetic acid at 0.3 μg/ml gave a consistent degree of inhibition throughout a wide range of template DNA concentrations whether DNA was below or above the saturation concentration (Table 1). These results indicate that inhibition from phosphonoacetic acid was not due to binding of this compound to the template.

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