The DNA Polymerases of Vaccinia Virus-infected Animal Cells

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Increased DNA polymerase (E.C. 2.7.7.7) activity after infection with vaccinia virus has been found in several different host cell systems (Magee, 1962; Green, Piña & Chagoya, 1964; Jungwirth & Joklik, 1965). Various lines of evidence indicate that this increase results from the formation of a new DNA polymerase (Jungwirth & Joklik, 1965; Magee & Miller, 1967; Berns, Silverman & Weissbach, 1969).

We report here that the enzyme produced in two different infected-cell systems has similar properties, and can in each case be distinguished from the DNA polymerase of the uninfected cells. This suggests that the genetic information for the post-infection DNA polymerase is encoded in the virus genome, rather than in a normally repressed portion of the host cell genome.

L cells were grown in suspension as described by Gold & Helleiner (1964). Primary explants of chick embryo fibroblasts were prepared by the method of Paul (1965). Vaccinia virus (strain L1-3), supplied by Dr K. Rozee, Microbiology Dept, Dalhousie University, was grown in L cells and partially purified by centrifugation. L cells were infected with 3 to 5 p.f.u./cell and chick cells with 5 to 8 p.f.u./cell. In each case, a large majority of cells was infected, as judged by counting colonies of surviving cells or by infectious centre assay. In all experiments reported, substantial multiplication of virus occurred.

L cells were harvested and re-suspended in cold water at 2 to 3 x 10^7 cells/ml. To each 1 ml. of cell suspension were added 0.05 ml. of 0.1 M-KHCO₃ + K₂CO₃ buffer (pH 10) and 0.05 ml. of 0.1 M-EDTA (potassium salt, pH 10). The mixtures were kept in ice and periodically stirred. After 15 min. the highly viscous preparation was centrifuged at 37,000g for 20 min. The supernatant fluid was dialysed for 12 hr against 4 l. of buffer A (Gold & Helleiner, 1964) containing 10 % (v/v) glycerol. Chick cell extracts were prepared in the same way after scraping cells into cold phosphate-buffered saline. A final concentration of 0.01 M-bicarbonate buffer was needed for lysis of chick cells.

The standard reaction mixture for DNA polymerase contained in 0.25 ml.: 200 µg. of heat-denatured calf thymus DNA, 2 µmoles MgCl₂, 2 µmoles 2-mercaptoethanol, 50 nmoles of each of dATP, dGTP, dCTP and TTP, and up to 0.5 mg. of cell extract protein in buffer A. Except where otherwise indicated, potassium phosphate concentration was adjusted to give 13.6 µmoles of phosphate (25 μatoms of potassium). [α-³²P]TTP was added to give a final specific activity of about 10⁸ counts/min./nmole. After 60 min. at 37°C the samples were assayed for acid-insoluble radioactivity by the method of Bollum (1968). A unit of DNA polymerase activity is defined as the incorporation of 1 nmole/hr of TTP into DNA.

Increases in DNA polymerase activity after infection were greater in stationary than in exponentially growing L cells. Activities of 4.0 to 4.9 units/mg. protein in uninfected stationary-phase L cells increased to 10.1 to 18.4 units/mg. protein 5 hr after infection. Activities in confluent uninfected chick cells of 1.2 to 1.5 units/mg. protein increased to 2.8 to 5.4 units/mg. protein 5 hr after infection. Extracts were used within 3 days of preparation, during which time no loss in activity occurred. Over longer periods of storage, infected-cell DNA polymerase was considerably more stable than control L-cell enzyme. For example, after 20 days at 2°C, the remaining activity of a control L-cell preparation was only
20\%, but that of an infected L-cell preparation was 89\%. Glycerol markedly retarded the loss of activity in all preparations.

The effects of varying the concentration of K\(^+\) (supplied as potassium phosphate buffer) on the activities in four different types of extract were compared (Fig. 1). The DNA polymerases from the two kinds of uninfected cells were clearly distinguishable (Fig. 1a), but the DNA polymerases of the infected cells, themselves indistinguishable, differed from those of either of the two uninfected host cells (Fig. 1b).

![Graph 1](image1.png)

**Fig. 1.** Effect of potassium ion concentration on DNA polymerase activity in extracts of vaccinia-infected and uninfected cells. (a) Uninfected cells; (b) vaccinia-infected cells. 0--0, L cells; (3--0, chick cells.

The heat sensitivity of the different DNA polymerases was compared by preincubating extracts at 42° in the presence of 25\% glycerol on the DNA polymerase activity in extracts of vaccinia-infected and uninfected cells. (a) Uninfected cells; (b) vaccinia-infected cells. 0--0, L cells; (3--0, chick cells.

![Graph 2](image2.png)

**Fig. 2.** Effect of preincubation at 42° in the presence of 25\% glycerol on the DNA polymerase activity in extracts of vaccinia-infected and uninfected cells. (a) Uninfected cells; (b) vaccinia-infected cells. 0--0, L cells; (3--0, chick cells.

The heat sensitivity of the different DNA polymerases was compared by preincubating extracts at 42° in the presence of glycerol (25\%, v/v). Samples were withdrawn periodically and assayed at 37° without removing the glycerol. The stability of the enzymes was independent of protein concentration or the initial level of activity, and in contrast to the findings of Keir et al. (1966) with DNA polymerase from herpes-infected cells, inclusion of DNA in the preincubation mixtures had no effect on stability. Extracts from both types of uninfected cells lost activity on preincubation at 42°, the L-cell enzyme being inactivated more rapidly (Fig. 2a). Extracts of infected cells of both types contained a DNA polymerase
activity which was more stable and showed a slight tendency towards heat activation (Fig. 2b). The contribution of remaining host-cell enzyme could account for the apparently greater heat activation in infected chick-cell extracts than in L-cell extracts.

Several other properties of DNA polymerases were investigated but no consistent differences were found. All four extracts showed the same optimal Mg\(^{2+}\) concentration (8 to 20 mM) in the presence of either native or denatured DNA, and similar saturating concentrations of substrates and DNA primer; activity with native DNA as primer was 10 to 20% of that with denatured DNA.

Various attempts were made to distinguish the DNA polymerases of infected and uninfected L cells by separating them. Of these, **fractionation with (NH\(_4\))_2SO\(_4\) was the most successful.** Dialysed extracts were brought to the required % saturation with solid (NH\(_4\))_2SO\(_4\) and centrifuged. The precipitates were redissolved in and dialysed against buffer A containing 10% glycerol (v/v) before assay (Table 1). The DNA polymerase from vaccinia-infected L cells was consistently precipitated at lower concentrations of (NH\(_4\))\(_2\)SO\(_4\), and could be recovered in good yield in the fraction precipitating between 15 and 40% saturation. The K\(^+\) activation curve for the fraction precipitating at 20% saturation resembled that of the original infected-cell extract, while that for the fraction precipitating between 20 and 40% saturation resembled that of an uninfected-cell extract. Since the completion of this work, the separation of DNA polymerase from vaccinia-infected and uninfected HeLa cells has been reported by Berns et al. (1969).

**Table 1. Fractionation of L cell DNA polymerases with (NH\(_4\))\(_2\)SO\(_4\)**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Uninfected</th>
<th></th>
<th>Vaccinia-infected</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific</td>
<td>Total</td>
<td>Specific</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>activity</td>
<td>activity</td>
<td>activity</td>
<td>activity</td>
</tr>
<tr>
<td>Crude cell extract</td>
<td>1.35</td>
<td>25.0</td>
<td>5.6</td>
<td>95.6</td>
</tr>
<tr>
<td>0-20% saturation</td>
<td>0.15</td>
<td>0.9</td>
<td>7.4</td>
<td>19.6</td>
</tr>
<tr>
<td>20-40% saturation</td>
<td>3.8</td>
<td>17.1</td>
<td>4.2</td>
<td>20.2</td>
</tr>
</tbody>
</table>

Total activity is expressed in units, the specific activity in units/mg. protein.

Our results indicate that a new DNA polymerase is present in vaccinia-infected L cells and chick cells, in agreement with the conclusions of others for infected HeLa cells. Because of the identity of the new enzyme in the two different host cells, our results suggest that the source of information for the structure of the new DNA polymerase is probably the virus genome itself. Strong support for this suggestion is given by observations on the temperature sensitivity of the DNA polymerase of the closely related variola virus (Bedson & Cruickshank, 1969). More rigorous proof awaits the discovery of conditional lethal mutants of vaccinia virus with an impaired DNA polymerase.

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REFERENCES


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