Characterization of DNA Polymerase Induced by Salmon Herpesvirus, *Oncorhynchus masou* Virus

By SATORU SUZUKI, TAKAHISA KIMURA and MINEO SANEYOSHI*

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060

1Faculty of Fisheries, Hokkaido University, Hakodate 041, Japan

(Received 6 November 1985)

**SUMMARY**

A DNA polymerase induced by *Oncorhynchus masou* virus (OMV) was isolated from cultured salmon cells infected with OMV using sequential ion-exchange column chromatography steps. The properties of the OMV polymerase were compared to those of human cytomegalovirus (HCMV) polymerase and polymerase alpha from cherry salmon (masu salmon, *O. masou*) testes. OMV polymerase was clearly distinguished from the other polymerases by its optimum temperature for enzyme activity, 25 °C. In an investigation of the effects of phosphonoacetic acid and aphidicolin, OMV polymerase was found to be more resistant to both drugs than HCMV polymerase.

*Oncorhynchus masou* virus (OMV) is a pathogenic herpesvirus of salmonid fish (Kimura et al., 1981a) and is oncogenic in salmonid fish (Kimura et al., 1981b). Although it is known that herpes group viruses such as herpes simplex virus (HSV) (Keir & Gold, 1963; Weissbach et al., 1973; Purifoy, 1975/6; Powell & Purifoy, 1977; Knopf, 1979; Mar & Huang, 1979; Ostrander & Cheng, 1980), human cytomegalovirus (HCMV) (Huang, 1975; Miller & Rapp, 1976; Nishiyama et al., 1983), varicella-zoster virus (Miller & Rapp, 1977; Mar et al., 1978), Epstein-Barr virus (Miller et al., 1977; Ooka et al., 1979), herpesvirus saimiri (O’Hare & Honess, 1983) and Marek’s disease herpesvirus (Boezi et al., 1974) induce unique viral DNA polymerases in the infected cell, there has been no report to date concerning DNA polymerase induction by a herpesvirus of poikilotherms. In this study, we isolated the OMV polymerase from infected cells and compared its properties with those of other DNA polymerases, i.e. DNA polymerase alpha from salmon testes and HCMV polymerase. We found that OMV polymerase showed optimum activity at a low temperature (25 °C) and that the enzyme was more resistant to inhibition by phosphonoacetic acid (PAA) or aphidicolin than HCMV polymerase.

For the isolation of viral polymerase, OMV-infected kokanee salmon (*O. nerka*) ovary cells (KO-6) (Lannan et al., 1984) were used as a source of enzyme. Confluent monolayers of KO-6 cells were infected with OMV at a multiplicity of approximately 2 p.f.u./cell. Incubation was performed at 15 °C, which was the optimum temperature for virus replication, and assays were performed as described by Yamaguchi et al. (1980). Time course studies revealed that a novel DNA polymerase activity, which could be detected in the presence of 100 mM-ammonium sulphate, increased in virus-infected cells, and maximum activity was observed 48 h after virus infection. KO-6 cells infected with OMV were harvested 48 h after infection and were sonicated in hypotonic buffer containing 50 mM-Tris-HCl pH 7.5, 200 mM-KCl, 1 mM-EDTA, 10 mM-2-mercaptoethanol (2-ME), 20% (v/v) glycerol, 0.5 mM-phenylmethylsulphonyl fluoride and 0.5% (v/v) Triton X-100. The resulting homogenates were centrifuged at 22000 g for 120 min. The supernatant fluid was applied to DEAE-cellulose (Whatman DE-52) after dialysis against buffer A (20 mM-Tris-HCl pH 7.5, 1 mM-EDTA, 10 mM-2-ME, 20% glycerol). The column was washed with buffer A containing 20 mM-KCl, and then eluted with a linear gradient from 20 mM- to 0.5...
M-KCl in buffer A. Polymerase activity detected by assay in the presence of 100 mm-ammonium sulphate was eluted between 0.2 M and 0.25 M-KCl (Fig. 1b). Since this activity was not observed in the case of mock-infected cells (Fig. 1a), this novel polymerase was considered to be OMV-induced. In the next step, the pooled OMV polymerase fraction (fractions 45 to 56) from the DEAE-cellulose column was applied to a phosphocellulose column (Whatman P-11) after dialysis against 20 mM-KCl in buffer A. The column was washed and eluted with a linear KCl gradient from 20 mM to 0.6 M in buffer A. OMV polymerase activity was eluted as a single peak at 0.47 M-KCl (Fig. 1c). After the chromatography steps, OMV polymerase exhibited a specific activity of 374 units/mg, and was purified 23-fold from the centrifuge supernatant. Viral polymerase was dialysed against 50 mM-Tris–HCl pH 7.5, containing 10 mM-2-ME, 1 mM-EDTA, 150 mM-KCl and 60% glycerol and stored at −20 °C. HCMV polymerase was partially purified by the same procedure from human embryonic fibroblasts infected with HCMV Ad169.

The effect of salts on the polymerase activity is shown in Fig. 2. OMV polymerase activity was stimulated maximally twofold at 250 mM-KCl and 3.5-fold at 100 mM-ammonium sulphate. Using NaCl, no remarkable stimulation was observed. These properties are similar to those of HSV polymerase (Weissbach et al., 1973; Purifoy, 1975/6; Powell & Purifoy, 1977; Knopf, 1979).
and HCMV polymerase (Miller & Rapp, 1976; Nishiyama et al., 1983). It was found that polymerase alpha from salmon testes was inhibited by ammonium sulphate (Fig. 2b).

The temperature dependence of the activity of the various polymerases was studied. As shown in Fig. 3(a), OMV polymerase had an optimum temperature of 25 °C, and retained 50% of the activity even at 10 °C. Similar findings have been reported for the reverse transcriptase of the retrovirus of northern pike (Esox lucius) (Papas et al., 1976). They reported that the optimum temperature for a mammalian retrovirus enzyme was 30 °C, that of an avian retrovirus enzyme was 38 °C, while the pike retrovirus enzyme had an optimum temperature of 20 °C. In this paper, the correlation between the optimum temperature for herpesvirus DNA polymerase activity and the host suggests a long association of the virus with the host. Heat inactivation of OMV and HCMV polymerases was examined. Each enzyme was exposed to various temperatures for 20 min, then the enzyme solution was cooled in an ice-bath and was added to the standard assay mixture. Incubation was performed at 25 °C for 20 min. The results (Fig. 3b) indicate that OMV polymerase was inactivated to 15% of the control value by exposure to 30 °C for 20 min. From these experiments, it was concluded that the OMV polymerase had its optimum activity at a lower temperature and was more thermolabile than HCMV polymerase.

OMV polymerase resembled HCMV polymerase in other properties such as optimum pH and requirement for divalent cations (Table 1). Partially purified OMV polymerase showed

### Table 1. Comparison of the properties of DNA polymerases

<table>
<thead>
<tr>
<th>Property</th>
<th>OMV polymerase</th>
<th>HCMV polymerase</th>
<th>Polymerase alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum pH</td>
<td>7.5–8.0</td>
<td>7.5–8.0</td>
<td>8.0*</td>
</tr>
<tr>
<td>Mg$^{2+}$ (mM)</td>
<td>2.5–5.0</td>
<td>2.5–5.0</td>
<td>2.5–5.0</td>
</tr>
<tr>
<td>Mn$^{2+}$ (mM)</td>
<td>60</td>
<td>60</td>
<td>No effect</td>
</tr>
<tr>
<td>$K_m$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dATP (μM)</td>
<td>4.7</td>
<td>2.86</td>
<td>1.1*</td>
</tr>
<tr>
<td>dGTP (μM)</td>
<td>3</td>
<td>ND†</td>
<td>0.52*</td>
</tr>
<tr>
<td>dCTP (μM)</td>
<td>2.4</td>
<td>ND</td>
<td>0.71*</td>
</tr>
<tr>
<td>dTTP (μM)</td>
<td>5.6</td>
<td>3.77</td>
<td>2.4*</td>
</tr>
<tr>
<td>50% Inhibition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAA (μM)</td>
<td>28</td>
<td>5</td>
<td>38</td>
</tr>
<tr>
<td>Aphidicolin (μg/ml)</td>
<td>2.25</td>
<td>1.75</td>
<td>3.25</td>
</tr>
</tbody>
</table>

* S. Nishimura et al. (unpublished data).
† ND. Not done.

Fig. 3. (a) Optimum temperatures for the activity of herpesvirus polymerases and polymerase alpha. (b) Heat stability of OMV polymerase and HCMV polymerase (see text). O, OMV polymerase; ●, HCMV polymerase; △, HSV-2 polymerase; ▲, polymerase alpha.
somewhat higher \( K_m \) values for deoxyribonucleotides than did HCMV polymerase. OMV polymerase was more resistant to PAA and aphidicolin than HCMV polymerase. This may be reflected in the higher \( K_m \) values for substrates. However, to assess the susceptibility of the enzyme to inhibitors more precisely, further purification of the viral enzymes used here would be required.

In conclusion, we have isolated and characterized the DNA polymerase from a poikilothermic herpesvirus. The OMV polymerase had an optimum temperature of 25 °C, retained 50% of its activity even at 10 °C, and it was relatively resistant to PAA and aphidicolin in comparison with HCMV polymerase.

We would like to thank Dr M. Yoshimizu, Hokkaido University, for his valuable suggestions and technical assistance. This work was supported in part by a Grant-in-Aid for Cancer Research (58010094) from the Ministry of Education, Science and Culture, Japan to M.S.

REFERENCES


(Received 19 June 1985)