Immunochemical Characterization of Pyrimidine Kinase Induced by Varicella-Zoster Virus

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SUMMARY

Thymidine kinase (TK) induced by varicella-zoster virus (VZV) was precipitated with ammonium sulphate and purified by Sephadex G-150, QAE-Sephadex and Blue Sepharose column chromatographies. The purified TK fraction also contained deoxycytidine kinase (dCK) activity and a 35000 mol. wt. (35K) polypeptide as a major component. The TK and dCK activities were both neutralized by anti-VZV serum. Antiserum to an extract of cells infected with a bromodeoxyuridine (B UdR)-resistant mutant virus contained no antibody to the viral TK and dCK activities or to the 35K polypeptide. Antiserum to the purified viral TK fraction was prepared and absorbed with a lysate of BuDR-resistant mutant virus-infected cells. The resulting absorbed antiserum (anti-vTK serum) neutralized the viral activities of both TK and dCK, and specifically immunoprecipitated a 35K polypeptide from the lysate of parental virus-infected cells, but did not immunoprecipitate any detectable polypeptide from cells infected with BuDR-resistant mutant virus. Anti-vTK serum stained mainly the nuclei of cells infected with the parental virus strain, but did not stain those infected with BuDR-resistant mutant virus by an indirect fluorescent antibody test. These results suggest that the 35K polypeptide produced in VZV-infected cells is responsible for the viral TK and dCK activities, and that the TK and dCK are mainly localized in the nuclei of infected cells.

INTRODUCTION

Thymidine kinase (TK) induced in cells infected with herpes simplex virus (HSV) has been studied extensively. Biochemical, genetical and immunological experiments have suggested that the genome of HSV codes for its own TK (Dubbs & Kit, 1964; Ogino et al., 1973; Jamieson & Subak-Sharpe, 1974; Honess & Watson, 1974a, b). Recently, the nucleotide sequence of the TK gene in the HSV genome has been determined (McKnight, 1980; Swain & Galloway, 1983) and the molecular weight of the HSV enzyme was estimated as 40000 (40K) (Summers et al., 1975; Kit et al., 1978; Sanders et al., 1982; Marsden et al., 1983). Since HSV TK can use both thymidine and deoxycytidine as substrates, it is also called deoxypyrimidine kinase (Jamieson & Subak-Sharpe, 1974).

Varicella-zoster virus (VZV), another member of the herpesvirus group, has also been reported to induce TK and deoxycytidine kinase (dCK) activities on its infection of cells (Dobersen et al., 1976; Ogino et al., 1977; Hackstadt & Mallavia, 1978; Cheng et al., 1979). These reports suggested that VZV codes for the induced TK and dCK and that a single enzyme has both activities, as reported for HSV. Dobersen et al. (1976) and Cheng et al. (1979) characterized the chromatographic behaviour of VZV deoxypyrimidine kinase by Sephadex G-150 and Blue Sepharose column chromatography, respectively. In this work, we purified VZV deoxypyrimidine kinase by a combination of the above procedures and QAE-Sephadex column chromatography. The purified enzyme was characterized immunologically, and it was shown
that VZV TK with a mol. wt. of 35K was associated with dCK activity. Moreover, immunofluorescence staining with antiviral thymidine kinase (vTK) serum showed that VZV TK was mainly located in the nuclei of VZV-infected cells.

**METHODS**

**Cells.** Human embryonic lung (HEL) cells were grown in a mixture of equal amounts of Eagle's MEM and Medium 199 supplemented with 10% calf serum and maintained in the same medium with 3% calf serum.

**Viruses.** The plaque-purified Kawaguchi strain and Oka strain of VZV (Takahashi et al., 1975) were used as parental strains. Bromodeoxyuridine (BUDR)-resistant mutants were isolated as described previously (Shiraki et al., 1983). Briefly, the parent virus was infected onto HEL cells and passaged serially in the presence of increasing concentrations of BUDR. Then the mutant was plaque-purified twice in the presence of 10 μg/ml BUDR. Mutants were isolated in independent experiments. The mutant from the Kawaguchi strain was designated as BUDR-R-K, and the mutants from the Oka strain were designated as BUDR-R-O1, BUDR-R-O2, BUDR-R-O3, BUDR-R-O4 and BUDR-R-O5. They were sufficiently resistant to BUDR to show the same virus titre in the absence and presence of 10 μg/ml BUDR, and they did not induce viral thymidine kinase activity in infected cells.

**Thymidine kinase and deoxycytidine kinase assay.** TK and dCK activities were assayed by the method of Ogino et al. (1977). When more than 70% of the monolayer showed a cytopathic effect (c.p.e.), the cells were washed three times with phosphate-buffered saline (PBS) and treated with 0.02% EDTA in PBS. The resulting pellets were washed three times with PBS by low-speed centrifugation and suspended in 50 mM-Tris-HCl buffer pH 8.0 containing 150 mM-KCl and 3 mM-2-mercaptoethanol (2-ME) at a cell concentration of 5 × 10⁶ cells/ml, and then sonicated in an ice-bath. The sonicated samples were centrifuged at 20000 r.p.m. for 60 min in an RP-40 rotor (Hitachi) at 4 °C. The supernatants were used as crude enzyme extracts. An extract of mock-infected cells was prepared in the same way as the uninfected cell extract. The reaction mixture contained 0.2 μCi [14C]thymidine (sp. act. 57 mCi/mmol, Amersham) or 2 μCi [3H]thymidine (sp. act. 45 Ci/mmol, Amersham) for the TK assay, or 0.2 μCi [14C]deoxycytidine (sp. act. 487 mCi/mmol, Amersham) or 2 μCi [3H]deoxycytidine (sp. act. 54 Ci/mmol, Amersham) for the dCK assay, 5 mM-ATP, 5 mM-MgCl₂, the enzyme extract and 50 mM-Tris-HCl pH 8.0 to a total volume of 250 μl. The reaction was conducted at 38 °C for 15 min and was stopped by immersing the mixture in a boiling water-bath for 2 min. The amount of phosphorylated thymidine or deoxycytidine was determined by the DEAE-cellulose disc method. Neutralization of TK or dCK activity by antiserum was carried out by incubation of the enzyme extract with antiserum that had been dialysed against 50 mM-Tris-HCl buffer pH 8.0 containing 150 mM-KCl, for 1 h at 4 °C. The enzyme activity was linear with time for at least 30 min under the conditions used. The background counts in TK and dCK enzyme assays were subtracted.

**Purification of thymidine kinase.** The crude enzyme extract was fractionated with 20% to 50% saturations of ammonium sulphate as described by Cheng et al. (1979). The precipitate was solubilized in the buffer used for the Sephadex G-150 (Pharmacia) column (20 mM-Tris-HCl pH 8.0, 10% glycerol, 3 mM-2-ME) and subjected to Sephadex G-150 column chromatography as described by Dobersen et al. (1976). Elution of TK was monitored by assay of its activity. The peak fractions of TK activity were applied to a QAE-Sephadex (Pharmacia) column. TK was eluted with the same column buffer as for Sephadex G-150, but containing a linear gradient of 0 to 0.3 M-KCl. The eluted peak fractions of TK activity were extensively dialysed against the buffer used for Sephadex G-150 chromatography, at 4 °C. The dialysed fraction was applied to a Blue Sepharose (Pharmacia) column, which has been reported to separate viral TK from host cell enzymes (Cheng et al., 1979). The column was washed extensively with 0.45 M-Tris–HCl pH 7.5 and the material was eluted with 1.5 M-Tris–HCl pH 7.5 containing 300 μM-thymidine. The eluted TK activity was tested for ability to be neutralized by anti-VZV serum. The fractions with activity were precipitated with 10% TCA and the pellet was washed with ethanol and dried for analysis by SDS-PAGE. The gel was stained with silver stain (Daiichi Pure Chemicals Co., Tokyo, Japan). Protein concentration was determined as described by Bradford (1976).

**Production of antisera to VZV and viral thymidine kinase.** Anti-VZV green monkey serum was prepared by immunization of a green monkey with VZV-infected green monkey cells as described previously (Shiraki et al., 1982). The neutralizing antibody titre of the serum obtained was 1:1280.

Antiserum to vTK was prepared as follows. The purified TK fraction was inoculated with Freund's complete adjuvant into a rabbit three times at 3-week intervals, and serum was obtained 10 days after the last injection. This serum was absorbed with an enzyme extract of BUDR-R-K-infected cells and the absorbed serum was used as anti-vTK serum.

**Affinity column specific for BUDR-R-K-infected cells.** Antiserum to BUDR-R-K-infected cells was obtained as follows. BUDR-R-K-infected cells were sonicated and mixed with Freund's complete adjuvant, and then the mixture was inoculated into rabbits three times at 3-week intervals. Antiserum was obtained 10 days after the last injection. This serum antibody titre to VZV was more than 1:1280 in a neutralization test. An affinity column to BUDR-R-K-infected cells was prepared by conjugation of the γ-globulin fraction of anti-(BUDR-R-K-infected cell) serum to CNBr-activated Sepharose 4B as described previously (Shiraki et al., 1982).
**Immunoprecipitation and SDS-PAGE.** Immunoprecipitation and SDS-PAGE were done as described previously (Shiraki et al., 1982). Briefly, infected cells were labelled with 10 μCi/ml \[^{35}S\]methionine (sp. act. 1390 Ci/mmol, Amersham) in methionine-depleted Eagle's MEM containing 3% calf serum for 12 h after appearance of c.p.e. Cells were solubilized with TD buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.15 M NaCl, 1 mM phenylmethylsulphonyl fluoride in 20 mM-Tris-HCl buffer pH 7.5). The supernatants obtained after high-speed centrifugation (100000 g for 2 h) were immunoprecipitated with various sera and purified on Protein A-Sepharose (Pharmacia). The immune complexes obtained were analysed by SDS-PAGE and fluorography.

**Isolation of a polypeptide with viral TK and dCK activities.** The crude enzyme extract of cells infected with the Kawaguchi parent virus was chromatographed on an affinity column coupled with the γ-globulin fraction of anti-(BUdR-R-K-infected cell) serum. The applied and eluted fractions were assayed for TK and dCK activities and their polypeptide compositions were analysed by SDS-PAGE. The crude enzyme extract labelled with \[^{35}S\]methionine was also applied to the affinity column and the eluate was analysed for viral polypeptides by immunoprecipitation with anti-VZV serum and by SDS-PAGE and fluorography.

**Analysis of BUdR-resistant mutants (Oka strain).** The BUdR sensitivities of mutants of the Oka strain were determined by measuring the effective doses of BUdR for 50% plaque reduction (ED₅₀). The resistance was determined as the ratio of the virus titres in the absence and presence of BUdR at 10 μg/ml. Enzyme assay and immunoprecipitation were done as described above.

**Immunofluorescent study.** HEL cells were grown on coverslips in 60 mm glass plates. The cells were infected with the parent virus (Kawaguchi strain) and BUdR-R-K at an input multiplicity of infection of about 0.005. After appearance of c.p.e., cells were washed with PBS, air-dried and fixed in acetone for 5 min at −20 °C. Anti-vTK serum and preimmune serum were diluted 1:5 with PBS and then added to coverslips. After incubation at 37 °C for 30 min, the coverslips were washed thoroughly with PBS and incubated with fluorescein-conjugated anti-rabbit IgG serum diluted 1:20 (Cappel Laboratories). After a second incubation at 37 °C for 30 min, the coverslips were washed with PBS, mounted in a 1:9 solution of PBS and glycerine, and examined in a Nikon fluorescence microscope.

**RESULTS**

**Purification of TK from a crude enzyme extract**

TK was purified as described in Methods. The degree of purification is shown in Table 1. The purity was more than 1667-fold that of the ammonium sulphate fraction. The high degree of purification in the Blue Sepharose step was due to the affinity of TK for Blue Sepharose and its specific elution with thymidine. As shown in Table 2, the anti-VZV serum neutralized the VZV-

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Total protein* (mg)</th>
<th>Total activity* (c.p.m.)</th>
<th>Specific activity (c.p.m./mg protein)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ammonium sulphate</td>
<td>152</td>
<td>3.3 x 10⁷</td>
<td>0.21 x 10⁶</td>
<td>1</td>
</tr>
<tr>
<td>2. Sephadex G-150</td>
<td>17</td>
<td>2.5 x 10⁷</td>
<td>1.47 x 10⁶</td>
<td>7</td>
</tr>
<tr>
<td>3. QAE-Sephadex</td>
<td>2.43</td>
<td>2.0 x 10⁷</td>
<td>8.47 x 10⁵</td>
<td>39.2</td>
</tr>
<tr>
<td>4. Blue Sepharose</td>
<td>&lt;0.01</td>
<td>3.5 x 10⁶</td>
<td>&gt;350 x 10⁵</td>
<td>&gt;1667</td>
</tr>
</tbody>
</table>

* Total protein and total activity were calculated based on the volume at each step. The activity was assayed after dialysis against 50 mM-Tris-HCl buffer pH 8.0 containing 150 mM-KCl and 3 mM-2-mercaptoethanol.

**Table 2. Neutralization of TK and dCK activities with anti-VZV serum**

<table>
<thead>
<tr>
<th>Enzyme extract</th>
<th>TK activity (c.p.m.)</th>
<th>dCK activity (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-serum Post-serum</td>
<td>Pre-serum Post-serum</td>
</tr>
<tr>
<td>Uninfected HEL cells</td>
<td>2828 3029 (107)*</td>
<td>3495 3674 (105)</td>
</tr>
<tr>
<td>VZV-infected HEL cells</td>
<td>14024 2754 (19.6)</td>
<td>11978 3060 (25.5)</td>
</tr>
<tr>
<td></td>
<td>1891 2112 (112)</td>
<td>1904 2156 (113)</td>
</tr>
<tr>
<td>Purified TK fraction</td>
<td>2779 338 (12.2)</td>
<td>1615 270 (16.7)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are activities in the presence of post-serum as percentages of activities in the presence of pre-serum.
induced TK and dCK activities. This serum was used as anti-VZV serum in further analyses. The low level of phosphorylation of radioactive thymidine or deoxycytidine was due to competition with the high concentration of unlabelled thymidine used for elution from Blue Sepharose, not to low enzyme activity. The TK and dCK activities of the purified TK fraction were neutralized with anti-VZV serum and thus seemed to be viral activities (Table 2). The polypeptides in this fraction were analysed by SDS–PAGE. A 35K polypeptide was the most prominent, although other polypeptides were also present in this fraction (data not shown).

**Affinity chromatography of the infected cell extract on anti-(BUdR-R-K-infected cell extract) serum**

BUdR-R-K did not induce viral TK and dCK activities (Table 2; Shiraki et al., 1983) or a 35K polypeptide (Fig. 1a, lane 3) in infected cells. The crude enzyme extract from cells infected with the parent strain was subjected to affinity chromatography on a column coupled with anti-(BUdR-R-K-infected cell extract) serum. The total enzyme activities of TK and dCK were
Table 3. Neutralization of TK and dCK activities with anti-VZV serum before and after affinity chromatography coupled with anti-(BUdR-R-K-infected cell extract) serum*

<table>
<thead>
<tr>
<th>Serum (anti-VZV)</th>
<th>TK activity†</th>
<th>dCK activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Pre-serum</td>
<td>11916 (100)‡</td>
<td>3875 (100)</td>
</tr>
<tr>
<td>Post-serum</td>
<td>1590 (13.3)</td>
<td>372 (9.6)</td>
</tr>
</tbody>
</table>

* The affinity column was prepared as described in Methods.
† Enzyme activities were assayed in the presence of pre- or post-serum and are shown as c.p.m.
‡ Figures in parentheses indicate activities as percentages of that in the presence of pre-serum. The protein contents before and after chromatography were 3520 µg and 168 µg, respectively.

Table 4. Neutralization of TK and dCK activities with anti-vTK serum*

<table>
<thead>
<tr>
<th>Enzyme extract</th>
<th>TK activity (c.p.m.)</th>
<th>dCK activity (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-serum</td>
<td>Post-serum</td>
</tr>
<tr>
<td></td>
<td>Pre-serum</td>
<td>Post-serum</td>
</tr>
<tr>
<td>Uninfected HEL cells</td>
<td>1031 (100)</td>
<td>1382 (134)</td>
</tr>
<tr>
<td>Kawaguchi-infected HEL cells</td>
<td>52475 (100)</td>
<td>7833 (149)</td>
</tr>
</tbody>
</table>

* Enzyme activities were assayed in the presence of pre-serum and post-serum as described in Methods. Figures in parentheses indicate percentages of the activity in the presence of pre-serum.

calculated from the applied and eluted volumes. The recoveries of activities were calculated from the total enzyme activities applied and eluted. The recovery rate in the flow-through fraction from the applied activity was 92.6% for TK and 93.4% for dCK. These enzymes were shown to be viral by neutralization of their activities with anti-VZV serum (Table 3). In addition, immunoprecipitation by anti-VZV serum of [35S]methionine-labelled cell extract from cells infected with the parental virus revealed a 35K polypeptide as the only recognizable viral component in the flow-through fraction (Fig. 1b, lane 3). These results showed that the change in the BUdR-R-K mutant during selection with BUdR was accompanied at least by loss of the 35K polypeptide and that the viral enzyme activities (TK and dCK) were correlated with the viral 35K polypeptide.

Production of anti-vTK serum

Antiserum to the purified TK fraction was prepared and absorbed with BUdR-R-K-infected cell extract, as described in Methods. This absorbed serum (anti-vTK serum) neutralized the TK and dCK activities induced by the Kawaguchi parent virus, but not the cellular enzyme activities (Table 4). The anti-vTK serum was used for analysis of infected cells.

Analysis of a VZV-infected cell extract with anti-vTK serum

The profiles on SDS-polyacrylamide gels of immunoprecipitates with anti-vTK serum or anti-VZV serum of [35S]methionine-labelled extracts of cells infected with the parent strain or with BUdR-R-K are shown in Fig. 2(a). Only one band, corresponding to a molecular weight of 35K, was observed in the immunoprecipitate with anti-vTK serum of the extract of the infected parent strain (lane 6). No band was detected in extracts of uninfected (lane 3) or BUdR-R-K-infected (lane 9) with anti-vTK serum, or of uninfected cells (lane 2) or cells infected with the parent strain (lane 5) or BUdR-R-K (lane 8) treated with preimmune serum. Anti-VZV serum, which contained antibody to TK and dCK activities, immunoprecipitated many viral polypeptides, including the 35K polypeptide in the extract of the infected parent strain (lane 4), but not in extracts from uninfected (lane 1) or BUdR-R-K-infected cells (lane 7). Thus, again, a correlation of enzyme activities and the 35K polypeptide was shown.

Analysis of BUdR-resistant mutants of the Oka strain

Five BUdR-resistant mutants of the Oka strain were isolated and characterized (Table 5). None of them could induce viral TK and dCK activities in infected cells. PAGE analysis of
Fig. 2. SDS-PAGE of radioimmunoprecipitates of the VZV-infected HEL cell extract with anti-vTK or anti-VZV serum. (a) Extracts of cells infected with the Kawaguchi strain. Lanes 1 to 3, uninfected cells; lanes 4 to 6, cells infected with parental strain; lanes 7 to 9, BUdR-R-K-infected. Cell extracts labelled with $^{35}$Smethionine were analysed by SDS-PAGE after immunoprecipitation with anti-vTK (lanes 3, 6, 9), pre-serum (lanes 2, 5, 8) or anti-VZV serum (lanes 1, 4, 7). The arrow indicates 35K polypeptide. (b) Extract of Oka strain-infected cells. Lanes 1 and 2, extracts of cells infected with parent strain: lanes 3 and 4, BUdR-O1; lanes 5 and 6, BUdR-O2; lanes 7 and 8, BUdR-O3; lanes 9 and 10, BUdR-O4; lanes 11 and 12, BUdR-O5. Extracts labelled with $^{35}$Smethionine were analysed by SDS-PAGE after immunoprecipitation with anti-vTK serum (lanes 2, 4, 6, 8, 10, 12) or anti-VZV serum (lanes 1, 3, 5, 7, 9, 11). The arrow indicates 35K polypeptide.

Table 5. Analysis of BUdR-resistant mutants of the Oka strain

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Dose (µg/ml)</th>
<th>Ratio (%)</th>
<th>TK activity (c.p.m.)</th>
<th>dCK activity (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-serum</td>
<td>Anti-VZV serum</td>
<td>Pre-serum</td>
<td>Anti-VZV serum</td>
</tr>
<tr>
<td>Parent</td>
<td>1.1</td>
<td>29030</td>
<td>1616 (5.6)</td>
<td>18369</td>
</tr>
<tr>
<td>BUdR-R-O1</td>
<td>&gt;10</td>
<td>98.8</td>
<td>943 (82.7)</td>
<td>2161</td>
</tr>
<tr>
<td>BUdR-R-O2</td>
<td>&gt;10</td>
<td>98.2</td>
<td>1186 (107.0)</td>
<td>2344</td>
</tr>
<tr>
<td>BUdR-R-O3</td>
<td>&gt;10</td>
<td>93.7</td>
<td>1216 (115.4)</td>
<td>3000</td>
</tr>
<tr>
<td>BUdR-R-O4</td>
<td>&gt;10</td>
<td>96.2</td>
<td>1157 (1102)</td>
<td>2995</td>
</tr>
<tr>
<td>BUdR-R-O5</td>
<td>&gt;10</td>
<td>89.2</td>
<td>1499 (1382)</td>
<td>3373</td>
</tr>
</tbody>
</table>

* Virus titre in the presence of 10 µg/ml BUdR as a percentage of that in the absence of BUdR.
† Figures in parentheses show activities in the presence of post-serum as percentages of those in the presence of pre-serum.

radioimmunoprecipitates of extracts of mutant-infected cells with anti-vTK serum revealed the absence of the 35K polypeptide (Fig. 2b). Thus, a correlation of enzyme activities with the 35K polypeptide was also observed in another strain (Oka).

Immunofluorescent antibody staining with anti-vTK serum

The locations of TK and dCK in VZV-infected cells were examined by a fluorescent antibody test with anti-vTK serum (Fig. 3). Granular fluorescence was observed mainly in the nucleus and also weakly in the cytoplasm of parent strain virus-infected cells (Fig. 3a). No specific
fluorescence was observed in BUdR-R-K-infected cells (Fig. 3b) or uninfected cells. Preimmune serum did not stain any infected cells.

**DISCUSSION**

The molecular weight of the HSV type 1 TK polypeptide has been determined as 40K to 44K by electrophoretic analysis of lysates of cells infected with the wild-type or TK-deficient mutant virus (Honess & Watson, 1974a; Summers et al., 1975; Kit et al., 1978; Sanders et al., 1982). Thouless & Wildy (1975) identified a similar-sized polypeptide (42K) in cells infected with HSV type 2. The HSV TK polypeptide predicted from the TK nucleotide sequence has 376 amino acid residues and a mol. wt. of about 40K (McKnight, 1980; Swain & Galloway, 1983).

Recently, Preston & McGeoch (1981) identified two polypeptides with apparent mol. wt. of 43K (major polypeptide) and 39K (minor polypeptide) encoded by the HSV-1 TK gene both \textit{in vitro} (infected cells) and \textit{in vitro} (cell-free translation system). They suggested that these 43K and 39K polypeptides were translated from the same coding frame by use of a first and a second initiation codon. Furthermore, a small amount of a third polypeptide of 38K was also found to be translated \textit{in vitro} from the TK gene by use of a third initiation codon (Marsden \textit{et al.}, 1983). The 43K polypeptide was concluded to be the main TK polypeptide. The functional significance of the other two polypeptides in HSV-infected cells remains to be elucidated.

Studies on VZV have been retarded by its cell-associated nature. Dobersen \textit{et al.} (1976) estimated the mol. wt. of VZV TK as 70K by gel filtration, and Hackstadt & Mallavia (1978) estimated it as 72K by non-denaturing electrophoresis. Lopetegui \textit{et al.} (1983) suggested that a 35K mol. wt. polypeptide is a VZV TK from comparison of the polypeptides in cells infected with the wild-type Kawaguchi strain and the TK-deficient Kanno strain, and from the presence of this polypeptide in mouse cells transformed biochemically by VZV. The discrepancy in the estimated molecular weight could be explained by supposing that the active enzyme consists of two subunits of similar or identical size, as reported for HSV TK (Ogino \textit{et al.}, 1973; Honess & Watson, 1974b).
The high correlation of viral TK and dCK activities, and the correlation of these enzymic activities with the presence of a 35K polypeptide were shown in this study by the following results. First, the purified TK fraction had viral dCK activity and contained the 35K polypeptide as a major component. Second, BUdR-R-K did not induce the viral TK and dCK activities or the 35K polypeptide in infected cells, a finding which was confirmed on an affinity column coupled with anti-(BUdR-R-K-infected cells) serum. Infections of cells with five BUdR-resistant mutants of the Oka strain isolated independently also did not result in induction of viral TK and dCK activities or the 35K polypeptide. Third, anti-vTK serum specifically immunoprecipitated the 35K polypeptide and neutralized the viral TK and dCK activities induced by infection with the parent VZV. From these findings, it is suggested that viral TK and dCK are due to a single protein with a mol. wt. of 35K. Although it can not be ruled out that this enzyme is a minor polypeptide and another 35K polypeptide co-migrates with it, this seems to be the first polypeptide of VZV whose function has been elucidated. VZV TK is similar to HSV TK in molecular weight and in its association with dCK.

Mutants of HSV that were unable to induce TK activity have been reported (Dubbs & Kit, 1964; Buchan et al., 1970). Some of them were reported to induce synthesis of smaller novel polypeptides of various sizes related to intact viral TK with a mol. wt. of 42K (Cremer et al., 1978, 1979; Smiley et al., 1980). None of the six VZV mutants examined here could induce TK or the 35K polypeptide or related polypeptides in infected cells as judged by studies with anti-vTK serum. Therefore, they correspond phenotypically to the former TK deletion mutants of HSV. We did not find the latter mutants with a TK-related polypeptide, probably because our selection conditions were too strict to permit isolation of mutants with remnant TK activity.

In a fluorescent antibody test anti-vTK serum stained mainly the nuclei of infected cells. This suggests that viral pyrimidine kinase is located mainly in the nuclei of infected cells. From studies on biochemically transformed cells, Kit et al. (1980a,b) speculated that herpesvirus-associated nuclear antigen(s) (HANA) and HSV type 1 TK are identical or closely related. Yamanishi et al. (1981) detected nuclear fluorescence with anti-VZV serum in VZV biochemically transformed mouse cells. Our observation of the localization of TK in nuclei of infected cells is consistent with these findings on biochemically transformed cells.

REFERENCES


*(Received 15 May 1984)*