Deoxypyrimidine Kinases of Herpes Simplex Viruses Types 1 and 2: comparison of Serological and Structural Properties

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SUMMARY

The kinetics of formation, the stability at 40 °C and the serological properties of thymidine kinase and deoxycytidine kinase activities induced by herpes simplex virus have been examined. The results are consistent with the hypothesis that both activities are carried on the same molecule – a deoxypyrimidine kinase.

Mutants deficient in deoxypyrimidine kinase have been used to produce, by absorption of general antisera, deoxypyrimidine kinase-specific antisera. Using immunoprecipitation and SDS-polyacrylamide gel electrophoresis, only one size of polypeptide (mol. wt. 42 400 ± 200) has been found, constituting the type 2 enzyme.

This is close to published values for the type 1 enzyme but co-electrophoresis demonstrated that the polypeptide of the type 1 enzyme was slightly bigger.

INTRODUCTION

Hay et al. (1971) and Jamieson, Gentry & Subak-Sharpe (1974) have shown that herpes simplex virus mutants resistant to either BUdR or Ara C lack the ability to induce both thymidine kinase and deoxycytidine kinase activities. The latter authors concluded that the most likely explanation was that both enzyme activities were located on the same molecule. Jamieson & Subak-Sharpe (1974) showed that the induction of both activities by herpes simplex virus type 1 (HSV-1) was coincident and that the two activities could not be separated by velocity sedimentation in sucrose gradients or by polyacrylamide gel electrophoresis. They also showed that there was substrate competition by thymidine of the deoxycytidine kinase activity. This constitutes good evidence that herpes simplex virus specifies a deoxypyrimidine kinase active on both substrates.

Herpes simplex virus type 2 (HSV-2) thymidine kinase activity is unstable at 40 °C whereas that of HSV-1 is stable (Thouless & Skinner, 1971). It is possible to discriminate between HSV-1 and HSV-2 thymidine kinase by neutralization with known anti-HSV-1 and -2 sera. Certain HSV-1 antisera stabilize HSV-2 thymidine kinase activity (Thouless, 1972). We were therefore interested to see how far these type differences applied to deoxycytidine kinase activity.

Honess & Watson (1974a) prepared immune precipitates of isotope-labelled HSV-1 thymidine kinase. On analysis by SDS polyacrylamide gel electrophoresis, they found only one size of polypeptide, mol. wt. 44 000. We were therefore interested to investigate the polypeptides of the HSV-2 enzyme and to compare them with those of HSV-1.

In this paper we report experiments comparing the kinetics of induction, the thermo-
stability and serological properties of HSV-1 and HSV-2 induced deoxypyrimidine kinases and their constituent polypeptides.

METHODS

Cell lines. BHK 21 CI3 cells, grown in supplemented Eagle's medium containing 10% tryptose phosphate broth and 10% calf serum (ETC) (Vantsis & Wildy, 1962) were used in the preparation of infected cell extracts for the experiments.

RK 13 cells (Beale, Christofinis & Furminger, 1963) were grown in the same medium (with rabbit serum substituted for calf serum) for the preparation of antigen for immunizing rabbits.

Virus strains. The following virus strains were used in this work: herpes virus type 1, strains HFEM and WAL; herpes virus type 2, strains BRY and 2037; herpes virus type 1, thymidine kinase deficient mutant B2006 (Dubbs & Kit, 1964), hereafter referred to as HSV-1 TK-; herpes virus type 2 thymidine kinase deficient mutant derived from strain BRY and hereafter referred to as HSV-2 TK- (Thouless, 1972). As might be expected (Hay et al. 1971; Jamieson et al. 1974), both these thymidine kinase deficient mutants also lacked the ability to induce deoxycytidine kinase. The results depicted in all figures were obtained with strains HFEM and BRY. Virus infectivity was assayed by the suspension method of Russell (1962) using a carboxymethyl cellulose overlay.

General antisera. Anti-type 1 sera 192 and 467 were raised using freeze-dried antigen prepared from RK 13 cells infected with HSV-1 strain HFEM. The antigen + Freund's incomplete adjuvant was injected intramuscularly according to the prolonged schedule described by Watson et al. (1966). Anti-type 2 serum 409 was prepared in a similar fashion, using strain BRY, but the antigen was stored at -70°C instead of being freeze-dried. Anti-type 2 serum 367 was prepared by lymph node injection of type-2 (BRY) antigen stabilized with anti-type 1 serum as previously described (Thouless, 1972). These antisera were obtained from the same rabbits used by Thouless (1972) but were derived from different bleeds.

Serum absorption. This was carried out as described by Buchan, Luff & Wallis (1970). About 10⁶ cells infected with either HSV-1 TK- or HSV-2 TK- were disrupted 17 h after infection and mixed with 1 ml of homologous type general antiserum (467 or 409). The mixture was shaken at 4 °C overnight. It was centrifuged at 100,000 g for 1 h under liquid paraffin and the supernatant fluid was concentrated to 1 ml by pressure dialysis.

Immunodiffusion tests. These were carried out in 1% agar (Watson et al. 1966). A pattern of six wells, equally spaced around a central well, each 8 mm in diam. and separated by 3 mm, was used. Each well was filled to capacity with antigen or antiserum (approx. 0.12 ml) and left at room temperature for 48 h for lines of precipitation to develop.

One-step growth experiment. BHK cells (2 x 10⁶ per time point) were infected with 10 p.f.u./cell of HSV-1 or HSV-2. They were stirred for 1 h at 37 °C, then centrifuged and resuspended in 5 ml of trypsin-versene solution and held at 37 °C, for 5 min to eliminate free virus. The infected cells were dispensed in bottles and incubated at 37 °C for the required number of hours, harvested, washed in PBS, resuspended in 1 ml of distilled water and ultrasonically disrupted before infectivity and enzyme titration. In these experiments enzyme assays were done on crude cell sonicate. Infectious centre assays were done on a duplicate bottle 4 h after infection to determine the number of cells actually infected and recovered.

Preparation of herpes-infected BHK cell extracts. BHK cells were infected with HSV-1 or HSV-2 at an input multiplicity of 10 to 15 p.f.u./cell and incubated for 7 or 17 h at 37 °C. They were then washed with phosphate buffered saline (PBS) and resuspended at 10⁶ cells/ml of distilled water. The cells were disrupted by ultrasonic vibration and centrifuged at
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100,000 g under liquid paraffin for 30 min. The layer between the pellet and the paraffin was taken off and stored in small volumes at -70 °C until required for enzyme assays.

Preparation of radioactively labelled infected cell extract. BHK cells (5 x 10⁶) were infected with 10 to 15 p.f.u./cell of HSV-1 or HSV-2 in ETC. After stirring for 1 h at 37 °C for virus adsorption, they were washed three times with amino acid free Eagle’s medium and dispensed in Winchester flasks containing either: (a) 100 ml of amino acid free Eagle’s medium supplemented with normal levels of methionine, histidine, inositol, tyrosine, threonine, arginine and tryptophane plus [³H]-labelled valine, lysine, leucine, phenylalanine (100 μCi of each). It also contained 3 ml of normal Eagle’s medium containing 10 % calf serum and 150 cc of CO₂ were added, or (b) 100 ml of amino acid free Eagle’s medium with normal levels of amino acids mentioned in (a) except for methionine. 230 μCi of [³⁵S]-methionine were added and 150 cc of CO₂.

These cells were harvested after 17 h and processed as described in the previous section.

Determination of thymidine kinase activity. Thymidine kinase activity was measured as described by Klemperer et al. (1967). The reaction mixture was made up in bulk. 0.21 ml was dispensed in each tube. Infected cell extract (0.04 ml), suitably diluted in tris-HCl + NaCl was added. The final 0.25 ml mixture was 0.02 M-phosphate buffer pH 6, 5 mM-MgCl₂, 5 mM-ATP, 10-6 μM-[²-¹⁴C]-thymidine and 5 to 40 μg of protein. The mixtures were incubated for 10 min at 37 °, boiled for 2 min and centrifuged at 2000 g for 10 min to remove denatured protein. Supernatant fluids (0.05 ml) were spotted on DEAE cellulose paper and washed 3 times in 1 mM-ammonium formate. The products of the reaction stuck to the paper and the radioactivity was measured in toluene scintillator in a liquid scintillation counter.

Determination of deoxycytidine kinase activity. Deoxycytidine kinase activity was assayed in a similar fashion, but the pH, substrate, length of incubation and method of counting were altered. The pH optimum for deoxycytidine kinase activity was found to be higher than that for thymidine kinase, between pH 7-0 and 8-0 for HSV-2 and between pH 8-0 and 8-6 for HSV-1. The final 0.25 ml of reaction mixture was 0.02 M-tris-HCl, pH 8-0, 5 mM-MgCl₂, 5 mM-ATP, 10 μM-deoxycytidine and 0.5 μCi of deoxy[⁵³H]-cytidine and < 200 μg of protein. The mixtures were assayed for 30 or 45 min at 37 °C, boiled for 2 min and centrifuged at 2000 g for 10 min to remove denatured protein. Fifty microlitre samples of the supernatant fluid were spotted on 1.5 cm² pieces of DEAE cellulose paper. They were washed 3 times in 1 mM ammonium formate and dried. The radioactively labelled product of the reaction was eluted with 0.5 ml of 0.5 M-NaCl and 10 ml of triton-toluene scintillator were added. The vials were shaken well and counted in the liquid scintillation counter.

40 °C thermostability test. HSV-1 or HSV-2 infected cell extract was assayed for thymidine and deoxycytidine kinase activity and reassayed after being held at 40 °C for appropriate periods.

Neutralization of deoxypyrimidine kinase activity. Equal-sized samples of infected cell extract and antiserum were mixed and assayed for thymidine and deoxycytidine kinase activity within 5 min of mixing on ice and again after 30 and 60 min at 25 °C. The infected cell extract was diluted so as to give 10,000 to 20,000 ct/min/assay with pre-immune serum for thymidine kinase. The length of incubation and the number of 50 μl samples per vial were adjusted to give the same range of ct/min/assay for deoxycytidine kinase activity.

Preparation of radiimmune precipitates. Radioactively labelled HSV-1 or HSV-2 infected cell extract was mixed with anti-HSV-1 or anti-HSV-2 deoxypyrimidine kinase specific serum in optimal proportions determined from an agar gel immunodiffusion test. The sera were prepared by exhaustively absorbing anti-HSV-1 or anti-HSV-2 serum with the homologous type of deoxypyrimidine kinase negative mutant. The antiserum antigen mixture, about 1 ml,
was left overnight in a conical tube at 4 °C. It was centrifuged at 4000 rev/min for 15 min in a bench centrifuge. The precipitate was washed six times in cold PBS. It was resuspended in 1 % SDS. The radioactivity was measured and a suitable volume mixed with an equal volume of 2 % SDS 1·0 M-urea and 0·2 % dithiothreitol and incubated at 80 °C for 10 min to dissociate the polypeptides.

Polyacrylamide gel electrophoresis. The electrophoretic system employed was the discontinuous buffer system of Ornstein (1964) and Davis (1964) modified as described by Dimmock & Watson (1969) for use with SDS in the analysis of dissociated polypeptides. In each experiment proteins of known mol. wt. were electrophoresed in parallel with the experimental gels for estimation of mol. wt. of unknowns. The log mol. wt. of separated marker proteins was inversely proportional to their electrophoretic mobility for the range of gel strengths employed with this buffer system (Honess & Watson, 1974a). The standards used were:

<table>
<thead>
<tr>
<th>Gel 1</th>
<th>BSA</th>
<th>mol. wt. of polypeptide 68 000</th>
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<tr>
<td></td>
<td>Fumarase</td>
<td>mol. wt. of polypeptide 48 000</td>
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<tr>
<td></td>
<td>Lysozyme</td>
<td>mol. wt. of polypeptide 14 500</td>
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<tr>
<td>Gel 2</td>
<td>Catalase</td>
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</tr>
<tr>
<td></td>
<td>Alcohol dehydrogenase</td>
<td>mol. wt. of polypeptide 41 000</td>
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The dissociated immune precipitates were electrophoresed on 7·5 % gels at 5 mA/gel. The analytical gels were sliced into 1 or 2 mm slices. The radioactive material was eluted with 'Soluene' (Packard Instrument Co. Ltd) and counted in toluene scintillator. The control gels with protein standards were stained with Coomassie brilliant blue (Dimmock & Watson, 1969).

Isotopes. All radioactive materials used in this work were obtained from the Radiochemical Centre, Amersham, Bucks., and were:

- [2-14C]-thymidine 59 to 62 mCi/mmol
- Deoxy-[5-3H]-cytidine 500 mCi/mmol
- L-[4,5-3H]-leucine 20 to 38 Ci/mmol
- L-[4,5(n)-3H]-lysine monohydrochloride 18·8 Ci/mmol
- L-phenyl[2,3-3H]-alanine 1 Ci/mmol
- L-[2,3-3H]-valine 19 to 31·6 Ci/mmol
- L-[35S]-methionine 180 Ci/mmol

RESULTS

Growth kinetics

Infectivity, thymidine kinase and deoxycytidine kinase activity in BHK 21 cells were measured 2, 4, 6, 8, 11, 14, and 24 h after infection with HSV-1 or HSV-2. For each sample, 2 × 10⁶ cells were infected. These resulted in 1·1 × 10⁷ HSV-1 infected cells and 4·8 × 10⁶ HSV-2 infected cells, based on the results of infectious centre assays.

Both enzyme activities began to increase between 2 and 4 h after infection and reached a maximum between 8 and 11 h. The increase in enzyme activities preceded the increase in infectivity by a few hours. The rate of increase was the same for both enzyme functions (Fig. 1).
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Fig. 1. One-step growth curve for thymidine and deoxycytidine kinase activities and for infectivity in (a) HSV-1 and (b) HSV-2 infected cells. •—•, infectivity log (p.f.u./cell); ▲—▲, thymidine kinase activity expressed as log (ct/min/assay); ▼—▼, deoxycytidine kinase activity expressed as log (ct/min/assay).

Stability at 40 °C

Samples of HSV-1 and HSV-2 infected cell extracts were held at 40 °C. At various times a 1/10 dilution was made for the assay of thymidine kinase activity and 40 μl undiluted material was assayed for deoxycytidine kinase activity.

The rate of inactivation of thymidine and deoxycytidine kinase activities were very similar for both types of HSV. Both enzymes were stable at 40 °C in the case of HSV-1 and unstable in the case of HSV-2 (Fig. 2).

When HSV-2 infected cell extract had been held at 40 °C for 1 h it no longer gave precipitin lines in an immunodiffusion test against an HSV-2 deoxypyrimidine kinase specific serum.

Neutralization and stabilization of HSV-2 deoxycytidine kinase with hyperimmune sera

Samples of pre-immune serum (409) or anti-type 1 serum (192) or anti-type 2 serum (409) were mixed with equal-sized samples of HSV-2 infected cell extracts. These were assayed for deoxycytidine kinase activity within 5 min of mixing on ice and again after 30 and 60 min at 25 °C.

The HSV-2 induced deoxycytidine kinase was virtually completely inactivated by anti-HSV-2 serum and was clearly stabilized by this anti-HSV-1 serum (Fig. 3). The results are similar to those found with HSV-2 thymidine kinase but more extreme (cf. Thouless, 1972, and Table 1).
Fig. 2. Comparison of the thermostability of HSV-1 and HSV-2 induced thymidine and deoxycytidine kinase at 40 °C. Samples of each type of infected cell extract were held at 40 °C. At various times a 1/10 dilution was made for a thymidine kinase assay (10 min at 37 °C) and 40 μl undiluted were assayed for deoxycytidine kinase activity (45 min at 37 °C). ○—○, HSV-1 thymidine kinase; □—□, HSV-1 deoxycytidine kinase; ■—■, HSV-2 thymidine kinase; □—□, HSV-2 deoxycytidine kinase.

Comparison of antiserum inhibition of HSV-1 and HSV-2 induced thymidine and deoxycytidine kinase activity

Further antiserum inhibition experiments were done, assaying for thymidine and deoxycytidine kinase simultaneously. Fifty microlitres of antiserum were mixed with 50 μl of infected cell extract. These were assayed within 5 min of mixing on ice for thymidine kinase and deoxycytidine kinase activities.

The infected cell extracts and antisera used are shown in Table I. The results for each pair of enzymes and any particular serum are similar though not numerically identical. They show that the anti-type I serum inhibited both HSV-1 enzymes more than HSV-2. Anti-HSV-2 serum 367 inhibited all the HSV-2 enzymes more than the HSV-1 enzymes. Anti-HSV-2 serum 409 gave the same result but was obviously a stronger serum having a greater inhibitory effect on all the enzymes.

Both the neutralizing and stabilizing effects of these sera were increased for HSV-2 deoxycytidine kinase compared with thymidine kinase. On the other hand, for HSV-1, it was not consistent whether there was a higher residual activity with thymidine or deoxycytidine kinase.

Neutralization of stabilized thymidine and deoxycytidine kinase activity

These experiments were done to ascertain whether anti-HSV-1 serum, which stabilized HSV-2 induced deoxypyrimidine kinases, interfered with neutralization of these enzymes by anti-HSV-2 serum. One-tenth of a ml of serum was placed in a small bottle, 0.1 ml of HSV-2 infected cell extract was added, without delay 0.1 ml of a second serum was added. Samples were taken within 5 min of mixing on ice and assayed for thymidine and deoxycytidine kinase activity. They were re-assayed after 30 and 60 min at 25 °C.

When anti-type I serum was added before pre-immune serum, both enzyme activities
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Fig. 3. Neutralization and stabilization of HSV-2-induced deoxycytidine kinase by anti-HSV-1 and anti-HSV-2 sera. ○—○, pre-immune (409); ■—■, anti-HSV-1 (192); △—△, anti-HSV-2 (409).

Table 1. Comparison of serum inhibition of HSV-1 and HSV-2 induced deoxycytidine and thymidine kinase

<table>
<thead>
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<th>HSV-2 strains</th>
<th>HSV-1 strains</th>
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<tr>
<td>2037</td>
<td>BRY</td>
</tr>
<tr>
<td>TK*</td>
<td>dCyK†</td>
</tr>
<tr>
<td>TK</td>
<td>dCyK</td>
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<tr>
<td>TK</td>
<td>dCyK</td>
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<td>TK</td>
<td>dCyK</td>
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<tr>
<td>TK</td>
<td>dCyK</td>
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<table>
<thead>
<tr>
<th>Serum</th>
<th>Anti-HSV-2 367</th>
<th>Anti-HSV-2 409</th>
<th>Anti-HSV-1 192</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK*</td>
<td>23</td>
<td>6</td>
<td>91</td>
</tr>
<tr>
<td>dCyK†</td>
<td>8</td>
<td>1</td>
<td>140</td>
</tr>
<tr>
<td>TK</td>
<td>33</td>
<td>8</td>
<td>91</td>
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<tr>
<td>dCyK</td>
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<td>TK</td>
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<td>36</td>
</tr>
<tr>
<td>dCyK</td>
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</tr>
<tr>
<td>TK</td>
<td>64</td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td>dCyK</td>
<td>82</td>
<td>29</td>
<td>48</td>
</tr>
</tbody>
</table>

These two enzyme assays were performed simultaneously on samples from the same enzyme-antiserum mixture. The numbers are residual activity of the enzymes expressed as a percentage of the activity of infected cell extract plus pre-immune serum.

* Thymidine kinase.
† Deoxycytidine kinase.

were stabilized (Fig. 4). When anti-type 2 serum was added first and pre-immune or anti-type 1 serum second, the enzyme activities were greatly inactivated. If anti-type 1 serum was added before anti-type 2 serum the activities were somewhat reduced compared with anti-type 1 serum and pre-immune serum, and slightly reduced compared with pre-immune serum alone. However, the extent of inactivation was small compared with the effect of adding anti-type 2 serum first. This showed that the stabilizing anti-type 1 serum competed and interfered with anti-type 2 serum in its reaction with HSV-2 induced thymidine and deoxycytidine kinase activities. Yet again, HSV-2 deoxycytidine kinase plus anti-type 1 serum gave a very much higher level of activity than the enzyme plus pre-immune serum.
Fig. 4. Neutralization of HSV-2 induced (a) thymidine kinase and (b) deoxycytidine kinase previously stabilized with anti-HSV-1 serum 192. Antiserum was placed in a bottle, infected cell extract was added and then a second sample of antiserum was added. The mixtures were assayed simultaneously for thymidine and deoxycytidine kinase activity. ○—○, pre-immune+pre-immune; ■—■, anti-HSV-1+pre-immune; ▲—▲, anti-HSV-1+anti-HSV-2; ◇—◇, anti-HSV-2+anti-HSV-1; △—△, anti-HSV-2+pre-immune.

The difference in behaviour of the two enzyme activities in this experiment cannot be ascribed to continuing serological activity during the two enzyme assays (10 and 45 min) since the assay results were linear with time of incubation over 45 min.

Polyacrylamide gel electrophoresis of immune precipitates prepared from anti-type 1 and 2 serum absorbed with the homologous type of deoxypyrimidine kinase deficient mutant

Deoxypyrimidine kinase-specific immune precipitates were prepared using absorbed antisera and either [3H]-amino acid-labelled HSV-2, or [35S]-amino acid-labelled HSV-1 infected cell extract. After washing, the precipitate was disrupted with SDS+urea+dithiothreitol for 10 min at 80 °C and electrophoresed on 7.5 % gel. The gel was sliced, the counts eluted in Soluene and counted in a toluene scintillator. Proteins of known mol. wt. were run in parallel and stained with Coomassie blue.

A total of four experiments have been done of which one is shown in Fig. 5. All four profiles showed 3 peaks, one of which is the refractile boundary. The mol. wt. estimations of the polypeptide in the central major peak were 43 150, 41 700, 43 150 and 41 700, giving a mean of 42 400 ± 200. The HSV-1 deoxypyrimidine kinase had only one size of polypeptide, mol. wt. 44 000 (Honess & Watson, 1974a). In order to determine whether the HSV-1 polypeptide was, in fact, slightly larger than HSV-2, [35S]-labelled HSV-1 and [3H]-labelled HSV-2 immune precipitates were electrophoresed on the same gel. The HSV-2 deoxypyrimidine polypeptide moved 1 mm further than the HSV-1 polypeptide corresponding to a mol. wt. of ~600 (Fig. 6). Repetition of this experiment with fresh reagents yielded the same result.
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DISCUSSION

The results of our experiments support the hypothesis (Jamieson et al. 1974; Jamieson & Subak-Sharpe, 1974) that HSV-1 and HSV-2 each specify a deoxypyrimidine kinase, i.e. an enzyme molecule with both thymidine and deoxycytidine kinase activities.

Our mutant, HSV-2-TK−, provides an additional example of a herpes virus strain selected by virtue of resistance to BUdR which is deficient in both activities.

Like Jamieson & Subak-Sharpe (1974), we found the kinetics of induction of both enzyme activities were similar for HSV-1 even though we noted far greater increases than they did. We found that the kinetics of induction were similar also for HSV-2. The thermostability of both enzyme activities was closely similar using a kinetic test at 40 °C. In this our results differ from those of Jamieson & Subak-Sharpe (1974) who found that the thymidine kinase activity of HSV-1 was considerably more stable than the deoxycytidine kinase activity at 45 °C.

Serological experiments gave similar, though not identical, results with thymidine kinase and deoxycytidine kinase activities. Both enzyme activities of HSV-2 were stabilized by the anti-HSV-1 serum (192) and this stabilization was accompanied by interference with
neutralization by HSV-2 specific sera. We should note that this is contrary to the preliminary result previously mentioned in discussion (Thouless, 1972). The type specificity of enzyme neutralization reported for thymidine kinase activity (Thouless, 1972) was found also for deoxycytidine kinase activity. Some aspects of the serological behaviour of the two enzyme activities are interesting. For example, the different patterns of stabilization (Fig. 4) were consistently found. We are uncertain whether this reflects differences inherent in the configuration of the enzyme molecules or some technical factor of trivial importance.

Honess & Watson (1974a) showed that HSV-1 deoxypyrimidine kinase was composed of polypeptides of one size (mol. wt. 44,000) when analysed by SDS-polyacrylamide electrophoresis of immunoprecipitates. Our results with HSV-2 deoxypyrimidine kinase indicated a closely similar size (mol. wt. 42,400). Co-electrophoresis of HSV-1 and HSV-2 deoxypyrimidine kinase polypeptides clearly indicates that the HSV-1 polypeptide was slightly larger than that of HSV-2. This could be accounted for either by an excess of 3 to 6 amino acids or by many amino acid substitutions.

Ogino, Shiman & Rapp (1973), using Sephadex G-100, have detected, in partly purified HSV-1, material activity possibly associated with monomers (mol. wt. 58,000), dimers (mol. wt. 97,000) and larger aggregates. With HSV-2 they found only one peak of activity (mol. wt. 45,000 to 60,000). More recently, Honess & Watson (1974b) estimated the size of the active HSV-1 thymidine kinase molecule using Sephadex G-200 exclusion chromatography of infected cell extracts. When treated with a non-ionic detergent, activity eluted at a mol. wt. of 70,000. Further, Jamieson & Subak-Sharpe (1974) obtained evidence from poly-

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**Fig. 6.** Polyacrylamide gel electrophoresis of a mixture of [\(^3\)H]-HSV-2 and [\(^3\)S]-HSV-2 immune precipitates. These were prepared from antiserum absorbed with the homologous types of thymidine kinase negative mutant and mixed with homologous type of infected cell extract appropriately labelled. The precipitates were disrupted with SDS at 80 °C prior to electrophoresis. ●—●, [\(^3\)H]-HSV-2 immune precipitate; ■—■, [\(^3\)S]-HSV-1 immune precipitate.
acrylamide gel electrophoresis and sucrose gradient velocity sedimentation, that HSV-1 induced thymidine and deoxycytidine kinase activities ran together in regions suggesting very large molecules or aggregates; the authors did not evaluate the probable mol. wt. of these activities. We are inclined to accept for the time being that the active enzymes are polymeric. If this were so, the thermal instability of the HSV-2 deoxypyrimidine kinase might be explained on the basis of disaggregation of subunits. We suppose that the HSV-1 antibody which stabilizes HSV-2 deoxypyrimidine kinase activity reacts with an antigenic determinant(s) bridging two or more subunits and hence stabilizes the molecule. Clearly, this must be a type-common antigenic determinant. Since the stabilizing antiserum (192) inhibits the neutralization of HSV-2 deoxypyrimidine kinase activity by homotypic antiserum, it follows that it must contain a type-common antibody species (possibly the stabilizing antibody) which reacts at a site sufficiently close to the neutralizing site(s) to inhibit neutralization by steric hindrance. By the same token, the type-specific neutralization of both HSV-1 and HSV-2 induced enzymes must involve sites near to the active enzyme site(s). As pointed out by Jamieson & Subak-Sharpe (1974), the competitive inhibition of deoxycytidine kinase activity by thymidine indicates that active sites for both activities are the same (or very closely located).

We believe that the evidence reported here supports the contention of the Glasgow workers that HSV-1 and HSV-2 each specify a deoxypyrimidine kinase able to phosphorylate both thymidine and deoxycytidine. Our results combined with those of Jamieson & Subak-Sharpe (1974) suggest an enzyme molecule (possibly polymeric) with only one kind of active site adjacent to, or on, which are type-specific antigenic determinants. Close to these there are type-common sites involved in the inhibition of type-specific neutralization of the HSV-2 enzyme. These may be the same as the stabilizing site which must also be type-common.*

We should like to thank our colleagues for many helpful discussions. In particular, we wish to thank Dr R. W. Honess and Dr A. Buchan for directly stimulating certain experiments and Mrs C. Pettitt for useful technical advice. We are grateful for the support provided by the M.R.C. and for general support afforded by The Shell International Petroleum Company Limited.

REFERENCES


* Note added in proof.

Since going to press with this paper a report has appeared (Kit, S., Jorgensen, G. N., Leung, W.-Ch., Trkula, D. & Dubbs, D. R., 1973/74, *Intervirology* 2, 299–311) showing the mol. wt. of thymidine kinase of HSV-1 and HSV-2 to be 81 300 and 77 700 as determined by analysis on glycerol gradients. This is a good approximation of a dimeric grouping of polypeptides of mol. wt. 42 000. The HSV-1 thymidine kinase has a larger mol. wt. than HSV-2 as was shown by us for the polypeptides alone.


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