Differences in the Properties of Thymidine Kinase Produced in Cells Infected with Type 1 and Type 2 Herpes Virus

(Accepted 5 May 1971)

Thymidine kinase activity is known to increase in BHK 21 cells infected with herpes simplex virus type 1 (Klemperer et al. 1967). Moreover, there is now evidence showing that this is a virus-specified enzyme (Klemperer et al. 1967; Buchan & Watson 1969; Buchan et al. 1970). We now report a similar increase in thymidine kinase activity in BHK 21 cells infected with herpes simplex virus type 2. The pH optima are the same for both enzymes. The growth kinetics are similar and the type 2 virus always induces at least as much enzyme activity as the type 1 and usually up to twice as much. There are striking differences in the stability and serological specificity of the two enzymes.

Infected BHK cell extracts were prepared and the thymidine kinase activity measured by the method of Klemperer et al. (1967). Briefly, BHK cells were infected with herpes simplex virus at a multiplicity of infection of 10 to 20 p.f.u./cell. They were incubated at 37°C for 7 hr, suspended in distilled water (10⁸ cells/ml.), ultrasonically disintegrated and centrifuged at 100,000 g for 30 min. The supernatant fluid was assayed for enzyme activity. The 0.25 ml. reaction mixture contained the sample, phosphate buffer pH 6, and a final concentration of 5 mM-MgCl₂, 5 mM-ATP and 10⁻⁶ μM-[2-¹⁴C]thymidine. The mixture was incubated for 10 min., boiled for 2 min. and centrifuged at 2000 g to remove denatured protein, and then 0.05 ml. was spotted on to DEAE cellulose paper and washed in ammonium formate. The product of the reaction stuck to the paper and the radioactivity was measured in a liquid scintillation counter.

Infected BHK cell extracts were prepared from HFEM, an established strain of herpes virus type 1, from four recent oral isolates, s2, s3, HIL and WAL and one older isolate, NASI-I. Infected BHK cell extracts were also prepared with G-LOV, an established type 2 strain, three recent genital isolates, G-BRY, G-PAR and G-3345 and two older isolates, G-2248 and G-2037. The two types were distinguished by their pock-forming ability on the chorioallantoic membrane of 10-day-old chick embryos. The oral isolates formed small grey haemorrhagic pocks, while the genital isolates formed large white gelatinous pocks. The types were also distinguished by reciprocal neutralization kinetics and confirmed for eight of the strains – HFEM, WAL, HIL, G-LOV, G-BRY, G-3345, G-2037, G-2248 – by an indirect surface immunofluorescent test (Geder & Skinner 1971).

The thymidine kinase in undiluted extracts of cells infected with herpes simplex type 1 is rather stable. It can be kept at 4°C for at least 1 hr. However, type 2 thymidine kinase cannot be kept satisfactorily even at 4°C; it must either be assayed at once or stored immediately at -70°C, where it retains its activity for long periods. It was possible to discriminate between the two types of enzyme by keeping the samples at 40°C for 1 hr. Nearly all of the type 1 thymidine kinase activity remained after 1 hr but only 3% or less of the type 2 activity (Table 1).

Another difference, possibly related to stability, was that type 2 enzyme preparations did not give a linear dose/activity curve on dilution, whereas type 1 did. Linearity could be restored by diluting in 0.5% bovine serum albumen or in an extract of uninfected BHK cells (the supernatant from 10⁸ BHK cells disrupted in 1 ml. of distilled water and centrifuged at
100,000 g for 30 min.). Various other substances – thymidine triphosphate, thymidine, mercaptoethanol, glycerol and dithiothreitol – were tested but none of them restored linearity on dilution. The type 2 enzyme was not protected from heat inactivation at 40° by thymidine triphosphate, by thymidine or by uninfected cell extract.

We attempted to compare the reciprocal effects of antisera to herpes type 1 and type 2 infected cells. The antiserum which we have at present, against cells infected with type 2, did not inactivate the type 1 or 2 thymidine kinase. The antisera against RK 13 cells infected with type 1 (Watson et al. 1966) inactivated type 1 (Buchan & Watson, 1969) but did not inactivate the type 2 enzyme and even appeared to stabilize it. When cells infected with type 2 virus were extracted in this type 1 antiserum, the thymidine kinase became much more heat-stable at 40° (about 30 % residual activity after 1 hr).

Table 1. *Residual thymidine kinase activity after incubation of infected cell extracts for 1 hr at 40°*

<table>
<thead>
<tr>
<th>Type</th>
<th>Residual activity (%)</th>
<th>Type 2</th>
<th>Residual activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFEM</td>
<td>87</td>
<td>G-BRY</td>
<td>0.1</td>
</tr>
<tr>
<td>WAL</td>
<td>96</td>
<td>G-LOV</td>
<td>1.0</td>
</tr>
<tr>
<td>S3</td>
<td>100</td>
<td>G-PAR</td>
<td>0.3</td>
</tr>
<tr>
<td>NASH</td>
<td>85</td>
<td>G-3345</td>
<td>3.0</td>
</tr>
<tr>
<td>HIL</td>
<td>91</td>
<td>G-2248</td>
<td>0.1</td>
</tr>
<tr>
<td>S2</td>
<td>89</td>
<td>G-2037</td>
<td>3.0</td>
</tr>
</tbody>
</table>

In summary, we have demonstrated that thymidine kinase produced in cells infected by six different strains of herpes simplex virus type 1 is stable at 40°, and that thymidine kinase produced in cells infected by six different strains of herpes simplex virus type 2 is unstable. We also found a non-linear dilution curve for the enzyme from type 2 infected cells, and we found a serological difference, in as much as the type 2 enzyme is resistant to the sera which inactivated type 1. Work is in progress to determine whether it is possible to raise an antiserum to cells infected with herpes type 2 which will specifically neutralize the type 2 thymidine kinase.

We should like to thank Professor P. Wildy and Dr A. Buchan for much useful help and criticism in the course of this work. We also wish to thank Dr A. J. Nahmias (Emory University School of Medicine, Atlanta, Georgia), Dr C. A. C. Ross (Ruchill Hospital, Glasgow) and Dr T. H. Flewett (East Birmingham Hospital, Birmingham) for sending us virus strains used in this work.

The work was supported by a grant from the United Birmingham Hospitals Endowment Fund.

Department of Virology  
University of Birmingham  
Birmingham, England  

Department of Virology and Department of Obstetrics and Gynaecology  
University of Birmingham  
Birmingham, England

M. E. THOULESS  
G. R. B. SKINNER
REFERENCES


(Received 31 March 1971)