Absence of a Requirement for Host Polypeptides in the Herpes Virus Thymidine Kinase

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SUMMARY

Active herpes simplex virus-specific thymidine kinase was precipitated with specific antiserum from extracts of infected BHK 21 cells which had been differently labelled with radioactive amino acids before and after infection. Analysis of the content of pre- and post-infection isotope in polypeptides separated electrophoretically from these immune-precipitates demonstrated the single, virus-specific, polypeptide labelled with post-infection label. No new polypeptide was detected with the pre-infection label and the low content of pre-infection isotope indicated the absence of any pre-formed host protein in the herpes thymidine kinase. The thymidine kinase specific antiserum, raised in rabbits against infected rabbit kidney cells, precipitated polypeptides of indistinguishable mol. wt. (44000) from BHK 21 cells and HEp-2 cells infected with the same strain of herpes simplex type I.

The evidence that a virus coded polypeptide must be contained in the virus specific thymidine kinase in cells infected with herpes simplex virus, albeit indirect, is now formidable. Thus, there are elevated levels of a kinetically (Klemperer et al. 1967) and immunologically (Buchan & Watson, 1969) distinct enzyme in the infected cell. The enzyme is type specific (Thouless & Skinner, 1971) and is not produced in cells infected with some mutant viruses (Dubbs & Kit, 1964). Moreover we have recently analysed the polypeptide composition of dissociated immune precipitates made by reacting an antiserum specific for the herpes thymidine kinase with infected cell extracts labelled with radioactive amino acids after infection (Honess & Watson, 1974). These experiments showed that the active enzyme contained only a single polypeptide synthesized after infection. They did not, however, eliminate a contribution from a pre-existing host polypeptide to form a mixed host-virus heteropolymer as the active unit. We have now investigated this possibility by examining the polypeptide composition of thymidine kinase immune precipitates made from extracts of cells labelled with [3H]-amino acids before infection and with [14C]-amino acids after infection. We have also compared the polypeptides of thymidine kinases synthesized after infection of BHK 21 and HEp-2 cells with the same virus strain.

We have used herpes simplex virus type-I HFEM strain (HSV-1 (HFEM)), and the thymidine kinase deficient mutant B2006 of Dubbs & Kit (1964). Procedures for high multiplicity infection of BHK 21 cells, labelling of infected and uninfected cells with radioactive amino acids, the purification of naked virus particles ('capsids') and the preparation of a high speed supernatant fraction and its use in immune precipitation reactions were as previously described (Robinson & Watson, 1971; Honess & Watson, 1974). The preparation and properties of rabbit hyperimmune antisera to HSV-1 (HFEM) infected rabbit kidney cells (anti-1) and uninfected BHK 21 cells (anti-BHK) were as described by Watson et al. (1966). Antisera of restricted specificity to the structural antigen Band II (anti-Band II) which contain type-common neutralizing antibody (Sim & Watson, 1973) and precipitate a
Table 1. Precipitation of proteins labelled before and after infection with hyperimmune antisera to host and virus specific components

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Pre-infection label $^3$H cf/min</th>
<th>Post-infection label $^{14}$C cf/min</th>
<th>Ratio $^{3}$H/$^{14}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-1</td>
<td>127600</td>
<td>369500</td>
<td>0.34</td>
</tr>
<tr>
<td>Anti-BHK</td>
<td>117750</td>
<td>34550</td>
<td>0.36</td>
</tr>
<tr>
<td>Anti-Band II</td>
<td>11835</td>
<td>16010</td>
<td>0.74</td>
</tr>
<tr>
<td>Anti-TK</td>
<td>8650</td>
<td>18220</td>
<td>0.48</td>
</tr>
</tbody>
</table>

major glycosylated polypeptide of 47000 mol. wt. (Honess & Watson, 1974) were prepared as reported by Watson (1969) and Watson & Wildy (1969). Antisera specific for the herpes virus thymidine kinase (anti-TK) were prepared by absorption of antisera to type-1 virus with excess of an extract of cells infected with the thymidine kinase deficient mutant virus (Buchan, Luff & Wallis, 1970).

Thymidine kinase activity was assayed by the method of Klemperer et al. (1967).

Before analysing the content of pre-infection label in thymidine kinase it was necessary to estimate the native mol. wt. of the active enzyme in order to deduce the minimum stoichiometry required of an essential component. Gel filtration of an untreated sample of infected cell supernatant fraction on a calibrated column of Sephadex G-200 (eluted with phosphate-buffered saline, 3 $\mu$M-thymidine) showed a peak containing all the recovered enzyme activity close to, and within, the column void vol. and having a modal mol. wt. in excess of 120000. However, pre-treatment of such enzyme preparations with 0.5 % Nonidet P40, which did not result in any loss of enzyme activity, converted the majority of the enzyme to a form eluting with an apparent mol. wt. of about 70000. Since a unit with an estimated mol. wt. of 70000 retained full enzyme activity in the presence of 0.5 % Nonidet any component continuously required for activity must be present at least once per 70000 mol. wt. unit.

Cultures containing $5 \times 10^8$ uninfected BHK 21 cells were labelled with 50 $\mu$Ci of each of four $^3$H-amino acids (leucine, lysine, phenylalanine and valine) for a 16 h interval prior to harvesting for infection. After this labelling interval the uninfected cells were thoroughly washed in medium without labelled precursors and were then infected with HSV-1 (HFEM) at an input multiplicity of 20 p.f.u./cell. After an absorption period of 1 h, cells were re-incubated with 15 $\mu$Ci of each of the four $^{14}$C-amino acids, leucine, lysine, phenylalanine and valine, at 37°C until 18 h post-inoculation. The infected cells were then harvested, disrupted by sonic vibration and the extract used for the preparation either of purified naked particles ('capsids') as described by Robinson & Watson (1971), or of a high speed supernatant fraction (sedimentation for 60 min at 35000 rev/min in the SW40 rotor of a Christ Omega II ultracentrifuge) for use in immune precipitation reactions.

The ratio of pre-infection to post-infection label ($^{3}$H/$^{14}$C) incorporated and present in the supernatant fraction was 2:08, and that in purified capsids was 0:06. Since the pre-infection label remaining in purified capsids co-migrated with the previously characterized structural polypeptides (Robinson & Watson, 1971) the ratio of 0:06 was used to estimate the amount of pre-infection label in a virus-specific component attributable to turnover alone, that is to the breakdown and reincorporation of pre-infection label into components synthesized specifically after infection. The amounts of pre- and post-infection isotopes precipitated by excess of anti-1 and anti-BHK, anti-Band II and anti-TK antisera from equal vol. of super-
Fig. 1. Polyacrylamide gel electrophoresis of the polypeptides precipitated by anti-Band II (a) and anti-TK (b) from infected cell supernatant fraction labelled with $^{3}H$-amino acids before infection (-----) and with $^{14}C$-amino acids after infection (●●●●●●●●●●). RB = refractile boundary; PR = position of phenol red marker. The annotations on the polypeptides precipitated by anti-Band II antiserum (Fig. 1b), i.e. IIa, IIb and IIc are the designations previously applied to the major (IIb) and minor (IIa, IIc) components precipitated by anti-Band II antiserum (Hones & Watson, 1974).

The annotations on the polypeptides precipitated by anti-Band II antiserum (Fig. 1b), i.e. IIa, IIb and IIc are the designations previously applied to the major (IIb) and minor (IIa, IIc) components precipitated by anti-Band II antiserum (Hones & Watson, 1974).
Table 2. The distribution of pre- and post-infection label in polypeptides separated by gel electrophoresis of anti-TK immune-precipitate and the derived molar ratios of virus and host cell components

<table>
<thead>
<tr>
<th>Component</th>
<th>Radioactivity in component</th>
<th>Ratio of 3H/14C</th>
<th>Mol of virus* component per mol of cell† component</th>
</tr>
</thead>
<tbody>
<tr>
<td>44,000 mol. wt. (fractions 31 to 40)</td>
<td>1678 5359</td>
<td>0.31</td>
<td>19</td>
</tr>
<tr>
<td>≤ 15,000 mol. wt. (fractions 71 to 75)</td>
<td>1004 526</td>
<td>1.90</td>
<td>1.7</td>
</tr>
</tbody>
</table>

From recovered 14C ct/min there are

\[ 3.4 \left( \frac{5359}{44000} \right) \left( \frac{526}{15000} \right) \]

mol of virus specific component of mol. wt. 44,000 per mol of virus-specific component of ≤ 15,000 mol. wt. and therefore there are 5.8 mol of the 44,000 virus component per mol of the 15,000 mol. wt. cell component.

* A virus component being defined as having a ratio of 3H/14C of 0.06.
† A host cell component being defined as having a ratio of 3H/14C of 5.0 (see text).

and re-incorporation. This result was to be expected since contamination of immune precipitates occurs by non-specific co-precipitation of labelled components (Honess & Watson, 1974). The data of Table 1 were therefore not sufficient to exclude the presence of a necessary host protein in the thymidine kinase. The immune precipitates were dissociated into their component polypeptides (by heating at 80 °C for 10 min in 1 % SDS, 0.1 % dithiothreitol, 0.5 M-urea, 0.05 M-tris-HCl, pH 7.0) and electrophoresed on 7.5 % polyacrylamide gel cylinders in the presence of SDS, as described by Dimmock & Watson, (1969). The highest observed ratio of 3H/14C in the electrophoretically separated polypeptides from the anti-BHK immune precipitate (3H/14C = 5.0) was considered an estimate of the ratio characteristic of a host polypeptide.

The profiles of polypeptides separated by polyacrylamide gel electrophoresis of the anti-Band II and anti-TK immune precipitates are shown in Fig. 1 (a), (b). The ratio of 3H/14C across the 47,000 mol. wt. Band II polypeptide IIb (fractions 30 to 37 of Fig. 1 a) was 0.33 and the ratio across the 44,000 mol. wt. thymidine kinase polypeptide (fractions 31 to 40 of Fig. 1 b) was 0.31 (Table 2). In neither profile was there an independently migrating component with a 3H/14C ratio characteristic of a host component. Although it may be argued by analogy with the behaviour of the components of the Band II precipitate that the presence of a specific host protein in thymidine kinase is unlikely, this possibility can be eliminated more effectively by a quantitative consideration of the data. Thus the molar ratios of virus to host components for these polypeptides may be calculated from the corresponding 3H/14C ratio, using the above figure of 0.06 for the 3H/14C ratio in a virus specific component uncontaminated with host components and the ratio of 3H/14C of 5.0 for a pure host component. If \( x \) is the molar fraction of virus specific component, the molar fraction of host component is \( 1 - x \) and

\[ 0.06x + 5(1-x) = 0.31 \]

giving \( x = 0.95 \). In other words, over the region of the 44,000 mol. wt. polypeptide, one molecule of a host component for every 19 molecules of virus-specific polypeptide would give the observed 3H/14C ratio.

Electrophoresis of immune precipitates in 14 % gels showed that the components which migrated with the refractile boundary in 7.5 % gels were a mixture of small mol. wt. (12,000 to 15,000) contaminants which were non-specifically represented in immune precipitates
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(Honess & Watson, 1974). However, for the purposes of this analysis these components have been regarded as possible specific components of the thymidine kinase. Calculating as before we find in this region an average of 1.7 mol of virus-specific polypeptide for each mol of host polypeptide. From recovered 14C ct/min there are 3.4 mol of virus-specific component of mol. wt. 44,000 for each mol of virus-specific component ≤ 15,000 mol. wt. (Table 2). There are, therefore, 5.8 mol (= 3.4 × 1.7) of the 44,000 virus component for each mol of the ≤ 15,000 mol. wt. cell component. Since a unit which is at most a dimer of the 44,000 mol. wt. component retains full activity there can be no requirement for a pre-existing host component in the herpes thymidine kinase. In fact the contribution of host components to the anti-TK immune precipitate is entirely compatible with previous estimates of the contribution of non-specific co-precipitation of host cell components and, by analogy, with the observations presented here on the reaction of antiserum to Band II with the same pre-labelled antigen preparations.

There remained the possibility that the enzyme was composed of two polypeptide chains which, although of the same or closely similar mol. wt. were of non-identical sequences, and that one of these components was a host polypeptide synthesized specifically after virus infection. The likelihood of this possibility was tested by a comparison of the polypeptides precipitated by anti-TK antiserum from an artificial mixture of the supernatant fractions from HSV-1 (HFEM) infected BHK 21 cells labelled with [3H]-amino acids and infected HEp-2 cells labelled with [14C]-amino acids. The electrophoretic analysis of this immune precipitate demonstrated co-migration of 3H and 14C labels as a single band with an estimated mol. wt. of 44,000 (other than the small mol. wt. components).

We consider that the data presented here indicate that there is no requirement for a host protein component as part of the thymidine kinase induced by herpes simplex virus. Further evidence for the homogeneity of the thymidine kinase polypeptide remains of some interest and an analysis of the native configuration of the enzyme seems necessary especially in view of recent data indicating that the herpes thymidine kinase may also bear a deoxycytidine kinase activity (Hay et al. 1971).

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