Identification of the Herpes Simplex Virus Protein Kinase as the Product of Viral Gene US3

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SUMMARY

Previous work has shown that a novel protein kinase is induced after infection of cultured cells with herpes simplex virus type 1 (HSV-1). Separately, it has been reported that the protein encoded by HSV-1 gene US3 shows similarity in its amino acid sequence to members of the protein kinase family of eukaryotes. We have investigated the possibility that these two observations are connected by preparing an antiserum to a synthetic oligopeptide corresponding to the carboxy-terminal eight amino acids of the US3 protein. This antiserum reacted on immunoblots with a polypeptide of apparent molecular weight 68 000 from extracts of cells which had been infected with HSV-1. The antiserum also reacted strongly with a 68 000 molecular weight species from a preparation of the novel HSV-1 protein kinase which had been extensively purified and resolved from other protein kinases. In addition, the purified preparation phosphorylated a protein species, also of 68 000 apparent molecular weight, when incubated with [γ-32P]ATP. These data are consistent with gene US3 encoding the novel protein kinase induced after infection of cells with HSV-1.

During lytic infection of cells by herpesviruses phosphorylation of both viral and cellular proteins occurs (Pereira et al., 1977; Marsden et al., 1978; Wilcox et al., 1980; Kennedy et al., 1981). It is possible that some of these phosphorylations might be catalysed by a protein kinase encoded by the virus, and there have been several different reports of activities that might correspond to a viral enzyme from cells infected with herpesviruses (Lemaster & Roizman, 1980; Blue & Stobbs, 1981; Michelson et al., 1984; Katan et al., 1985; Montalvo & Grose, 1986; Purves et al., 1986a). However, these various activities differ markedly in fundamental characteristics such as substrate specificity and subcellular location, and it seems likely that some are enzymes of the host cell. Indeed, cellular enzymes are known to be responsible for several of the protein phosphorylations that occur in infected cells (Stevely et al., 1985; Katan et al., 1986; Jakubowicz & Leader, 1987). The best candidate for a protein kinase encoded by herpes simplex virus (HSV) is that described by Purves et al. (1986a). This enzyme is induced in cells after infection with HSV type 1 (HSV-1) and is similar in substrate specificity to an enzyme detected in cells infected with pseudorabies virus (PRV) (Katan et al., 1985, 1986). It appears to be independent of known regulatory molecules and can utilize ATP (but not GTP) to phosphorylate certain seryl or threonyl residues of basic substrates such as protamine. Its substrate specificity, and the fact that it is active in 1 M-KCl, clearly distinguish it from known cellular enzymes and the other putative viral protein kinases mentioned above.

DNA sequencing studies have revealed that the US3 genes of HSV-1 and HSV-2 (McGeoch et al., 1985, 1987) and the corresponding gene of varicella-zoster virus (VZV) (Davison, 1983) encode proteins that are clearly homologous with members of the protein kinase family of eukaryotes (McGeoch & Davison, 1986). The polypeptides encoded by the US3 genes of HSV-1
and HSV-2 have predicted molecular weights of 53000 and the corresponding VZV gene product has a predicted molecular weight of 44000. These gene products have not previously been recognized as protein species in infected cells. In the work described in this communication we used an oligopeptide-induced antiserum specific for the HSV-1 US3 gene product to identify the protein from cells infected with HSV-1. We also showed that this antiserum reacts with the novel protein kinase induced after HSV-1 infection.

The peptide NH₂-Tyr-Cys-Leu-Pro-Leu-Phe-Gln-Gln-Lys-COOH (Cambridge Research Biochemicals, Cambridge, U.K.) corresponds to the C-terminal eight amino acids of the predicted US3 protein sequence plus an additional tyrosine residue which facilitated coupling of the peptide to bovine serum albumin (BSA) (Bassiri et al., 1979). Rabbits were immunized with the peptide–BSA conjugate as described previously (Frame et al., 1986) and anti-BSA antibodies were removed from the immune sera by incubation with BSA–Sepharose. The anti-peptide sera from two rabbits were combined and the mixture was designated anti-US3 serum (A/US3). This antiserum was used in immunoblotting experiments to detect the protein product of the US3 gene. Proteins extracted from BHK cells infected with 20 p.f.u./cell HSV-1 strain 17 for 18 h and labelled with 100 μCi/ml [35S]methionine were electrophoretically separated on a 10% SDS–polyacrylamide gel and transferred to nitrocellulose strips. Antigens immobilized on the nitrocellulose were detected by antibody and 125I-labelled Protein A. In Fig. 1, lanes 2 and 3, the product of the US3 gene is detected as a 68000 molecular weight polypeptide. The A/US3 serum reacted weakly with a lower molecular weight species of 50000 which is presumably related to the 68000 molecular weight polypeptide and may be a cleavage product. The apparent molecular weights of the proteins reactive with the US3-specific antiserum were estimated as described by Taylor et al. (1987). No reaction was seen when the antiserum was blotted against an uninfected cell extract.

The novel protein kinase induced by HSV-1 was purified from cytoplasmic extracts of BHK cells infected for 18 h with HSV-1 (10 p.f.u./cell), essentially as previously described for the enzyme induced by PRV (Purves et al., 1987). This entailed successive chromatography on DEAE–cellulose, TSK phenyl-5PW, threonine–Sepharose and protamine–agarose (Table 1). The purification used an exogenous substrate (protamine) for assay, and was specifically directed at a particular enzyme activity found to be present only in infected cells. The TSK phenyl-5PW and protamine–agarose steps respectively resolve the virus-induced protein kinase from casein kinase II and the active proteolytic fragment of protein kinase C, the major contaminating protein kinases present at the DEAE–cellulose stage (data not shown). It can be seen from Fig. 2(a) that, although the final preparation of the purified protein kinase was not homogeneous, it contained only two major species of apparent molecular weights 68000 and 61000 when analysed by SDS–polyacrylamide gel electrophoresis. This represents an extensive decrease in protein complexity. [The 94-fold increase in specific activity from the DEAE–cellulose stage (Table 1) almost certainly underestimates the protein purification because of the poor overall recovery of activity compared with the enzyme induced by PRV, a consequence of a much lower initial specific activity.] The final preparation of the protein kinase had a distinct and narrow substrate specificity when assayed with a range of model peptides (Purves et al., 1986b). Thus, we consider that there is only a single protein kinase present at the final stage of purification.

The A/US3 serum was used in immunoblotting experiments with material from stages (4) and (5) of the HSV-1 protein kinase purification and with a purified preparation of bovine protein kinase C as control. After the threonine–Sepharose stage (4) of the purification, a 68000 molecular weight protein was detected by the A/US3 serum at a low level (Fig. 3, lanes 5 and 6). After the protamine–agarose stage (5) the amount of the 68000 molecular weight species detected was considerably increased (Fig. 3, lanes 2 and 3) despite the fact that less protein from stage (5) was blotted (see legend to Fig. 3). The US3-specific antiserum showed no reactivity with purified bovine protein kinase C (Fig. 3, lanes 8 and 9). A number of unrelated anti-peptide sera, including that shown in Fig. 3 and directed against the IE110 polypeptide of HSV-1 (Perry et al., 1986), were used as controls and exhibited no reactivity with any of the protein kinase preparations. The increase in the amount of the 68000 molecular weight US3 product detected...
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Fig. 1. Identification of HSV-1 US3 gene product by immunoblotting. Extracts of BHK cells infected with HSV-1 (20 p.f.u./cell) for 18 h and labelled with 100 μCi/ml [35S]methionine were run on a 10% SDS-polyacrylamide gel, blotted on to nitrocellulose (650 μg protein/strip), blocked for 1 h at 37 °C with 3% gelatin in 20 mM-Tris-HCl, 0.5 M-NaCl pH 7.5. The strips were probed with A/US3 serum at a 1:2 dilution (lane 2), 1:10 dilution (lane 3) and normal rabbit serum at a 1:2 dilution (lane 4) at 4 °C for 16 h. The serum was diluted in 1 mM-Tris-HCl, 0.09% NaCl pH 7.5 (NT buffer) and 1% BSA. The strips were washed extensively in NT buffer containing 0.05% Tween 20 and 1% BSA and then incubated for 2 h at 37 °C with 5 × 10^5 c.p.m. of 125I-labelled Protein A in NT buffer with 3% BSA. The strips were washed extensively in NT buffer containing 0.05% Tween 20 and 1% KI at room temperature and dried for autoradiography. Lane 1 shows the blotted infected cell polypeptide profile. The molecular weights of known viral polypeptides are shown on the left hand side.

Fig. 2. Polyacrylamide gel analysis and phosphorylation of the protein kinase induced by HSV-1, at different stages of purification. (a) One-dimensional gel electrophoresis under denaturing conditions (Purves et al., 1987) was performed using protein from the different stages of purification listed in Table 1: (1), 50 μg; (2), 25 μg; (3), 25 μg; (4), 25 μg; (5), 5 μg. The gel was stained with Coomassie Brilliant Blue, and the positions of migration of proteins of known molecular weights are indicated. (b) Phosphorylation of 1 μg protein from stage (4) (lane 6) and 0.25 μg protein from stage (5) (lane 7) of the purification. The preparations were incubated with 10 μM-ATP and 5 μCi [γ-32P]ATP in a buffer containing 0.25 M-KCl, 20 mM-Tris-HCl pH 7.6, 20 mM-MgCl₂ and 10 mM-2-mercaptoethanol.

in the purified material after stage (5) correlates well with the increase in specific activity of the protein kinase induced by HSV-1. This, together with the absence of any other protein kinase in the final stage of the preparation, provides evidence that the enzyme is the product of the HSV US3 gene.

Autophosphorylation is a widespread feature of eukaryotic protein kinases, and it has previously been found that homogeneous preparations of the enzyme induced by PRV also
Fig. 3. Detection of the US3 gene product in purified preparations of the protein kinase induced by HSV-1. Protein kinase preparations at stages (4) (5 μg protein/strip; lanes 5 to 7) and (5) (1 μg protein/strip; lanes 2 to 4) of the purification scheme were blotted and probed with A/US3 serum at a 1:2 dilution (lanes 5 and 2) and a 1:10 dilution (lanes 6 and 3) and a 1:2 dilution (lanes 4 and 7) of control anti-oligopeptide serum against the viral immediate early polypeptide IE110. A purified protein kinase C (Parker et al., 1984) preparation was included as antigen control and probed with 1:2 and 1:10 dilutions of the A/US3 serum (lanes 8 and 9) and the control antiserum (lane 10). Lane 1 shows an infected cell polypeptide profile blotted from the same polyacrylamide gel.

Table 1. Partial purification of the protein kinase induced by HSV-1

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Enzyme activity (units*)</th>
<th>Recovery (%)</th>
<th>Specific activity (units/mg)</th>
<th>Purification (-fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Post-ribosomal supernatant</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(2) DEAE-cellulose</td>
<td>29</td>
<td>(100)</td>
<td>0.33</td>
<td>(1)</td>
</tr>
<tr>
<td>(3) TSK phenyl-5PW</td>
<td>5</td>
<td>37</td>
<td>1.3</td>
<td>4</td>
</tr>
<tr>
<td>(4) Threonine-Sepharose</td>
<td>3</td>
<td>22</td>
<td>14</td>
<td>42</td>
</tr>
<tr>
<td>(5) Protamine-agarose</td>
<td>0.7</td>
<td>5</td>
<td>3.1</td>
<td>94</td>
</tr>
</tbody>
</table>

* One unit protein kinase incorporates 1 nmol phosphate/min into protamine under standard assay conditions.

exhibit this characteristic (Purves et al., 1987). When material from stage (4) of the HSV-1 protein kinase purification was incubated with 10 μM-ATP containing 5 μCi [γ-32P]ATP a number of species were weakly phosphorylated. When the final preparation [stage (5)] of the HSV-1 protein kinase was incubated with the same concentration of ATP we were able to detect phosphorylation of a single major species of similar apparent molecular weight to that of the
immunoblotted species of Fig. 1 and 3 (Fig. 2b). We interpret this finding as representing autophosphorylation of the HSV-1 protein kinase.

Thus, determination of the DNA sequence of the HSV-1 US3 gene and recognition of similarity in protein sequence to protein kinases have enabled us to identify the product of this gene as the protein kinase previously characterized biochemically in cells after HSV-1 infection. The apparent molecular weight of 68000 for the US3 protein is considerably higher than the 53000 molecular weight predicted from the DNA sequence. Such discrepancies frequently occur between estimates of molecular weights of HSV proteins from polyacrylamide gels and the values determined by DNA sequence analysis (see, for instance, Perry et al., 1986; McGeoch et al., 1985). The use of a specific oligopeptide-induced antiserum combined with purification of the protein kinase from infected cells has allowed us to assign a well characterized enzyme function to a previously unrecognized HSV-1 gene product. Although the physiological substrate(s) and role of this enzyme during virus infection remain to be established, identification of the unique protein kinase as the product of gene US3 represents an important step towards elucidating its function.

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REFERENCES


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