Post-translational modification of the tegument proteins (VP13 and VP14) of herpes simplex virus type 1 by glycosylation and phosphorylation

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VP13 and VP14, major tegument proteins of herpes simplex virus type 1 (HSV-1) and the products of the UL47 gene, have been shown by partial proteolytic mapping to have closely related protein sequences. These proteins are phosphorylated in virus-infected cells, but not in preparations of purified virus. They also contain O-linked oligosaccharide units which include β-1,4-N-acetyl galactosamine residues, as demonstrated by the binding of Dolichos biflorus lectin. This modification was detected only in purified virus and appears to be restricted to VP13/14 and VP22, another HSV-1 tegument protein.

VP13/14 has been shown to be the product of the gene UL47 (Whittaker et al., 1991) and it shares amino acid homology with gp10, a glycosylated tegument protein of equine herpesvirus type 1 (EHV-1). A monospecific polyclonal antibody raised against VP14 also recognizes HSV-1 VP13, and this suggested that VP13 and VP14 are related polypeptides. The products of the UL47 gene have also been determined by McLean et al. (1990), who identified two protein products of M, 82000 and 81000. The product of the UL47 gene has been shown to modulate the α-trans-inducing factor-dependent induction of α genes (McKnight et al., 1987; Zhang et al., 1991), although the gene is considered to be non-essential for virus growth in tissue culture because mutants with a deletion of this gene grow to levels very similar to that of wild-type virus (Barker & Roizman, 1990; Zhang et al., 1991). The experiments reported here aim to confirm the relationship between VP13 and VP14, and to study their post-translational modification in more detail.

Initial attempts at solubilizing purified HSV-1 selectively relied upon the relative ease of removal of envelope proteins by solubilization with non-ionic detergents. Extraction of the resulting capsid/tegument structures with high ionic strength buffers (up to 2M-NaCl) resulted in the selective extraction of mainly VP13, 14, -16 and -22. Fig. 1 shows the results of a typical solubilization experiment. An overloaded lane (1) containing purified HSV-1 is shown deliberately to demonstrate minor polypeptides associated with purified virus. Lane 2 contains material released by treatment with 1% (v/v) NP40, which contains the major glycoproteins, gB, gC and gD, as well as a selection of low abundance polypeptides. Lane 3 contains material released from...
Fig. 1. Analysis of proteins extracted from purified HSV-1 on 9% SDS-polyacrylamide gels stained with Coomassie blue R-250. Virus purification and SDS-PAGE were carried out as described previously (Whittaker & Meredith, 1990). Lane 1 contains 40 μg of purified enveloped virus; lane 2 contains material containing an equal amount of virus solubilized using 1% NP40; lane 3 contains material released from the pellet produced after solubilization using 1% NP40, 1 M-NaCl; lane 4 contains residual insoluble material. The major HSV-1 structural proteins are indicated; closed symbols in lane 3 indicate polypeptides which are selectively extracted, open symbols indicate polypeptides enriched in other fractions.

Fig. 2. Partial proteolytic digests of VP13 (lanes 1, 3 and 5), VP14 (lanes 2, 4 and 6) and VP16 (lanes 7, 8 and 9) using either no enzyme (lanes 1, 2 and 7), 0.3 μg endoproteinase Glu-C (lanes 3, 4 and 8) or 0.1 μg chymotrypsin (lanes 5, 6 and 9). The location of peptides unique to either VP13 or VP14 is indicated by triangles.

Capsid/tegument structures, after treatment with 1% NP40, utilizing buffer of high ionic strength (1% NP40, 1 M-NaCl); it is clear that VP13, -14, -16 and -22 are solubilized by this procedure, although some material is still associated with the pellet (lane 4). No attempt has yet been made to characterize further the insoluble material in this fraction which co-precipitated with the major capsid protein (VP5).

The immunological cross-reactivity demonstrated for these two proteins (McLean et al., 1990; Whittaker et al., 1991) suggested that VP13 and VP14 are related. To confirm this, preparations of purified virus were solubilized with 1% (w/v) SDS, labelled with 125I-containing Bolton-Hunter reagent as described previously (Meredith et al., 1989) and separated by SDS-PAGE. Bands containing VP13, -14 and -16 were excised and subjected to partial proteolytic peptide mapping, essentially by the method of Cleveland et al. (1977). Fig. 2 shows an autoradiogram of the peptide digests produced. The lanes containing undigested material are over-exposed and demonstrate that a small amount of degradation of each sample has occurred, probably due to slight acid hydrolysis during the staining of the preparative gel to reveal the protein bands. Endoproteinase Glu-C and chymotrypsin produced a characteristic profile of peptides from VP13, -14 and -16. The profiles obtained for VP13 and -14 were nearly identical with few peptides of dissimilar mobility, whereas the profile obtained for VP16 shows a range of peptides of completely different mobility and relative abundance. These results suggest that VP13 and -14 have nearly identical amino acid
sequence. These two proteins will therefore be referred to as VP13/14.

To clarify whether this group of proteins is phosphorylated in preparations of purified virus, autoradiography was carried out on preparations of virus grown in the presence of $^{32}$Porthophosphate. We were unable to demonstrate any radioactivity associated with any structural protein despite loading up to 50 $\mu$g of total virus protein on a gel, and we therefore decided to investigate whether phosphorylated species were detectable in infected cell extracts. Immune precipitates of identical infected cell extracts labelled with $^{35}$Smethionine or $^{32}$Porthophosphate are shown in Fig. 3. The $^{32}$P marker lane was over-exposed but no obvious phosphorylated VP13/14 could be detected. Anti-VP13/14 antibody precipitated VP13/14, and small amounts of VP16 and VP22 from $^{35}$S-labelled cultures, and two low abundance bands of 79K and 65K from $^{32}$P-labelled cultures. The 65K band has a slightly greater $M_r$ than VP16 (62K using this gel system) and its origin is, as yet, unknown.

Owing to the homology between VP13/14 and gp10 of EHV we undertook studies to determine whether VP13/14 was glycosylated. Labelling cells with $^{14}$C- or $^{3}$Hglucosamine failed to reveal any glycosylation of VP13/14, either in infected cells or purified virus (data not shown), and, as an alternative approach, we decided to use biotinylated lectins as probes. Extracts of purified virus were probed, essentially by the method of Jackson & Tjian (1988), and with a wide range of biotinylated lectins specific for a range of carbohydrate groups. Fig. 4(a and b) shows the reaction obtained with *Dolichos biflorus* lectin [specific for the sugar N-acetyl glucosamine (GalNAc)] which bound only to those proteins, extracted from the virus using salt and detergent, of $M_r$ identical to those of VP13/14 and VP22. Binding was not competed by GalNAc, but was prevented by prior treatment with hexosaminidase, as described previously (Whittaker *et al.*, 1990). Digestion with this enzyme prevented biotinylated lectin binding, but this was not
due to proteolytic activity as the reaction of anti-VP13/14 and anti-VP22 antibodies with the digested material revealed no evidence of protease activity. Furthermore, the carbohydrate does not contribute significantly to the Mr of each protein because deglycosylation produced no detectable change.

The tegument proteins of HSV-1, designated VP13 and VP14, were extracted from virus preparations stripped of envelope and glycoproteins using buffers of neutral pH and high ionic strength. These proteins were therefore clearly associated with the remaining capsid and tegument proteins by ionic rather than hydrophobic interactions. The high ionic strength would enhance the association of proteins which are bound by hydrophobic interactions.

Partial proteolysis of HSV-1 VP13 and 14 using endoproteinase Glu-C and chymotrypsin revealed that the proteins must be very closely related in protein sequence and these data, in conjunction with the immunological cross-reactivity (Whittaker et al., 1991), showed that the two polypeptides must be products of the same gene. The UL47 gene contains two in-frame initiation codons (McGeoch et al., 1988) and it has been proposed that two polypeptides may be produced in a situation analogous to that of HSV-1 thymidine kinase (TK) (McLean et al., 1990). The gene sequence for HSV-1 TK contains three in-frame ATG codons (McKnight, 1980); two mRNA species have been detected (Preston & McGeoch, 1981) and polypeptides corresponding in size to the two forms have been detected in HSV-1-infected cells.

The identification of O-linked carbohydrate on VP13/14 and VP22, which contains α-1,4-GalNAc residues, is a novel finding for herpesvirus proteins. The removal of lectin binding by hexosaminidase cleavage is indicative of the lectin binding through sugar chains linked to the proteins. We have not been able to demonstrate incorporation of radioactive sugars into the proteins and the technique of sugar labelling using galactosyl transferase (Schindler et al., 1976) can not be used because the enzyme will not use α-1,4-GalNAc residues as a substrate. The quantity of carbohydrate is probably small because there is no apparent shift in Mr after enzymic deglycosylation. A 150000 Mr, cytagemgalovirus matrix protein has also been demonstrated to contain O-linked carbohydrate in short chains containing β-1,4-GlcNAc residues (Benko et al., 1988). The significance of this form of modification to virus structural proteins is as yet unclear, but it appears to be particularly prevalent in proteins involved in transcriptional control (Jackson & Tjian, 1988).

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References


Short communication


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