Lipoproteins of varicella-zoster virus

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Human fibroblast cells infected with varicella-zoster virus (VZV) showed a slight increase in lipoprotein synthesis, with the production of two major viral lipoproteins, as detected by radioimmunoprecipitation (RIP). Three bands of Mr 73000, 90000 and 97000 were identified as forms of the VZV gpl glycoprotein. All three incorporated both palmitic and myristic acid, and were shown by thin-layer chromatography to contain myristic, palmitic and stearic acids. A very strong band corresponding to 7000 Mr, which may represent the product of VZV gene 49, was detected after RIP and in VZV-infected cells, and was shown to contain almost entirely myristic acid. Several minor bands were also detected. The possible functions of the lipoproteins are discussed.

Varicella-zoster virus (VZV) is the causative agent of both chickenpox (varicella), resulting from primary infection, and shingles (zoster), resulting from reactivation of latent virus. The genome of the virus has recently been sequenced (Davison & Scott, 1986), but many of the mechanisms of virus pathogenesis are still poorly understood. VZV is known to possess many functions which require interaction with membranes (Heath, 1987). With other viruses such functions are known to involve viral lipoproteins (Schmidt, 1983; Schultz & Oroszlan, 1984), which also appear to be important in many stages of viral pathogenesis. With the human herpesviruses, lipoproteins have been identified only for herpes simplex virus type 1 (HSV-1), of which the gE glycoprotein is known to incorporate palmitic acid (Johnson & Spear, 1983), and the UL11 gene to encode a myristylated protein with an apparent Mr of 13000 to 16000 (MacLean et al., 1989). Up to 13 lipoproteins (of which up to nine also appear to be glycosylated) were observed in early work with virions of equine herpesvirus type 1 by chemical staining (Abodeely et al., 1971) or radiolabelling with [3H]choline (Perdue et al., 1974). Here we report an analysis of lipoprotein production in cells infected with VZV.

Human MRC-5 fibroblast cells (Flow Laboratories) were infected with a clinical isolate (H-551) of VZV. Trypsin-dispersed VZV-infected cell monolayers were used at an uninfected : infected cell ratio of 4 : 1; sterile medium was used for mock infection. Cells were incubated at 37 °C in Eagle's MEM plus 5 ~ foetal calf serum. Radiolabelling was done with [9,10(n)-3H]myristic acid or [9,10(n)-3H]palmitic acid (Amersham) at 500 μCi per 25 cm² monolayer from 24 to 72 h after infection, with D-[6-3H]glucosamine hydrochloride (Amersham) at 100 μCi per 25 cm² monolayer from 24 to 72 h after infection, or with L-[35S]methionine at 75 μCi per 25 cm² monolayer from 66 to 72 h after infection. After radiolabelling, monolayers were washed with Dulbecco's phosphate-buffered saline A (PBS) and either lysed in SDS sample buffer containing 2-mercaptoethanol for separation by SDS-PAGE (Laemmli, 1970), or prepared for immune precipitation by sonication in PBS plus 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium azide, 1 mM PMSF, 1 mM p-hydroxymercuribenzoate. For SDS-PAGE 10-5% acrylamide/0-32% bisacrylamide gels were used. For resolution of low Mr proteins (less than 10000 Mr), gels also contained 50% (w/v) sucrose, and were run using 20 mA of constant power (Chambers & Samson, 1982). After electrophoresis, gels were infiltrated for fluorography using 2,5-diphenyloxazole (Bonner & Laskey, 1974).

Overall, we observed a slight increase in the level of lipoprotein biosynthesis in VZV-infected cells relative to mock-infected cells, whereas the total amount of protein present in infected cells was not significantly altered (data not shown). Only two lipoproteins were observed that were clearly specific to VZV-infected cells, at 7000 and 5000 Mr, (Fig. 1). These were apparent from both myristic acid and palmitic acid radiolabelling, but appeared to incorporate primarily myristic acid and palmitic acid, respectively. It was clear that, as expected (Magee & Courtneidge, 1985; Olson et al., 1985), the two fatty acids radiolabelled different populations of proteins in both uninfected and VZV-infected cells.

For radioimmunoprecipitation (RIP), cell lysates were clarified by centrifugation at 85000 g for 1 h, and...
preadsorbed for 1 h with *Staphylococcus aureus* cells (Calbiochem) at 37 °C prior to RIP with an equal mixture of four highly reactive convalescent sera from patients with zoster (complement fixation titres 1024 to >2048), or with monoclonal antibody (MAb) 3B3 against VZV gpI glycoprotein (kindly supplied by Professor C. Grose). After reaction for 2 h at 37 °C, immune complexes were collected on *S. aureus* cells for 1 h at 37 °C and separated from non-reactive proteins by four washes in PBS plus 0·1% Tween-20. Immune complexes were solubilized in SDS sample buffer containing 2-mercaptoethanol prior to electrophoresis and fluorographic infiltration. From RIP of a VZV-infected cell lysate, three major virus-specific bands were observed on *S. aureus* cells for 1 h at 37 °C and separated from non-reactive proteins by four washes in PBS plus 0·1% Tween-20. Immune complexes were solubilized in SDS sample buffer containing 2-mercaptoethanol prior to electrophoresis and fluorographic infiltration. From RIP of a VZV-infected cell lysate, three major virus-specific bands were observed with myristic acid radiolabelling (Fig. 1, lanes 5 and 6), at *M* × 7000 and a doublet at 90000/97000 (gpI). Also observed was a minor band at *M* × 73000 (pgpI), a complex of five minor bands at *M* × 35000 to 42000. Two bands at *M* × 16000 and 28000 which were present with uninfected cell material were more intense with virus-infected cells. With palmitic acid (Fig. 1, lanes 7 and 8), the bands at *M* × 73000 (pgpI) and 90000/97000 (gpI) were clearly more intense than with myristic acid. Minor bands at *M* × 38000, 40000 and 42000 were observed, but the *M* × 5000 band observed in lysates of infected cells (Fig. 1, lane 4) was only very weakly apparent after RIP.

After RIP with a MAb against gpI, the non-glycosylated form (pgpI) was apparent at *M* × 73000 (Fig. 2, lane 8), whereas the glycosylated forms were observed at *M* × 90000/97000 (Fig. 2, lane 6). When fatty acid-radiolabelled antigen was radioimmunoprecipitated with MAb, after prolonged exposure faint bands were observed at *M* × 90000/97000 with palmitic acid (Fig. 2, lane 4), as well as extremely faint bands at *M* × 90000/97000 with myristic acid, and at *M* × 73000 with palmitic acid. These latter bands were visible on the original fluorogram, but were too faint to reproduce in the figure. Their presence was confirmed by densitometry using a Joyce-Loebl Chromoscan 3 integrating densitometer.

It is known that the 14-carbon myristic acid radiolabel can be incorporated as (16-carbon) palmitate after a biochemical conversion, and that the palmitic acid radiolabel can be similarly incorporated as myristate (Buss & Sefton, 1985; Hedo *et al.*, 1987), although the former (a chain elongation) seems to be more common (Olson *et al.*, 1985; Schmidt, 1984). Therefore it was necessary to determine the nature of the fatty acids bound to gpI. In order to do this, the fatty acids present were analysed directly (by reverse-phase thin-layer chromatography, RP-TLC) or indirectly (by assaying hydroxylamine-resistant fatty acid incorporation).
For the direct assay, sections of dried polyacrylamide gel containing the lipoprotein of interest were cut, rehydrated, and hydrolysed in vacuo for 16 h in 6 M-hydrochloric acid at 110 °C. After hydrolysis, fatty acids were extracted three times with n-hexane, and analysed by RP-TLC on Merck RP-18 plates using acetonitrile : acetic acid (1:1) (Schmidt et al., 1989). Unlabelled fatty acid standards visualized with 2,5-diphenylhexatriene (Sigma) were used to locate the corresponding regions of the sample lanes, which were then assayed by liquid scintillation counting (Table 1a). This method was also used to confirm the purity of fatty acid radiolabels. It was found that approximately half of the myristic acid radiolabel appeared to be incorporated in an unaltered form, while the remainder underwent chain elongation to palmitic and (18-carbon) stearic acids. With palmitic acid, elongation to stearic acid was observed, but no significant conversion to myristate was detected, a result which is in accord with those of Olson et al. (1985) and Schmidt (1984). However, it was clear from these data that gpl appeared to contain both myristic and palmitic acids, and also to contain significant levels of stearic acid. It has been reported that the latter is incorporated into a similar range of proteins as that of palmitic acid (Marinetti & Cattieu, 1982). By contrast, the 7000 Mr band, which incorporated primarily myristic acid, was shown by this method to contain 87.5% of the myristic acid radiolabel in the original form, while the remainder underwent chain elongation to palmitic and (18-carbon) stearic acids. With other lipoproteins, palmitic acid is known to be attached to multiple sites, primarily cysteine residues, along the polypeptide chain (Kaufman et al., 1984; Magee & Courtneidge, 1985; Schmidt & Schlesinger, 1980). From densitometric quantification of the myristic and palmitic acid radiolabelled immunoprecipitates, together with the results obtained by direct fatty acid analysis, it is clear that gpl contains higher levels of palmitic than myristic acid. With other lipoproteins, palmitic acid is known to be attached to multiple sites, primarily cysteine residues, along the polypeptide chain (Kaufman et al., 1984; Magee et al., 1984), whereas myristic acid is usually present only on N-terminal glycine residues (Kamps et al., 1985; Pellman et al., 1985; Towler et al., 1987). Our observations are in accordance with this.

For the indirect assay, polyacrylamide gels containing electrophoresed radioimmunoprecipitates were incubated for 20 h in 1 M-hydroxylamine pH 7-0 prior to fluorography. The resultant image was compared with that from a control gel which was similarly incubated in 1 M-Tris–HCl buffer pH 7-0, and the total intensity of individual bands on the two fluorograms was quantified by densitometry. Comparison of the two was used to determine the level of hydroxylamine-resistant incorporation (Table 1b). The thioester bonds which link palmitic and stearic acids (Schmidt, 1984; Schmidt et al., 1979) to the polypeptide are cleaved more readily by hydroxylamine than are the amide bonds which link the majority of myristic acid residues to the polypeptide, resulting in the preferential removal of palmitic acid, although some myristic acid is removed after exhaustive digestion (Olson et al., 1985). This method provides a useful control for the direct method. It was found (Table 1b) that hydroxylamine resulted in the removal of 72.4% of the myristic acid radiolabel, and 93.5% of the palmitic acid radiolabel. These results are in good agreement with those obtained by direct analysis of the fatty acid content of gpl, and provide further evidence of the presence of both myristic and palmitic acids in VZV gpl. The presence of fatty acids in the Mr 73000 band (Fig. 1, lanes 6 and 8) suggests that fatty acylation occurs before substantial glycosylation, as reported also for palmitoylation of the HSV-1 gE glycoprotein (Johnson & Spear, 1983) and other lipoglycoproteins (Duphny et al., 1981; Magee & Courtneidge, 1985; Schmidt & Schlesinger, 1980).

Table 1. Fatty acid content of VZV gpl and pgpl

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<tr>
<th>Radiolabel</th>
<th>[3H]Myristic acid (%)*</th>
<th>[3H]Palmitic acid (%)</th>
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<tr>
<td>(a) Fatty acid content by RP-TLC (%)*</td>
<td>Myristate 46.3 3.2</td>
<td>Palmitate 35.2 57.2</td>
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<tr>
<td>(b) Hydroxylamine-resistant incorporation (%)*</td>
<td>27.6 6.5</td>
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* Densitometry of hydroxylamine-treated gel/Tris-treated gel (see text).
gene encodes two cysteine residues in the transmembrane region, which are likely to represent palmitoylation sites.

For myristic acid, the most intense band by far was at Mr 7000. This does not correspond to any well characterized VZV protein, or to any known VZV gene product with an N-terminal glycine residue (Davison & Scott, 1986). However, gene 49 encodes a protein of 8907 Mr, with such a residue. This gene also encodes the other residues in this region which are optimal for myristylation, and is the VZV homologue of the UL11 gene of cytomegalovirus. Among the other human herpesviruses, cytomegalovirus is known to induce membrane permeability changes (Rugolo et al., 1986), which may involve viral myristoproteins (Cross et al., 1984; Kamps et al., 1985; Van der Valk et al., 1987). HSV is known to alter lipid metabolism in infected cells (Ben-Porat & Kaplan, 1971; Compton & Courtney, 1985), whereas VZV alters cellular lipid metabolism in a strain-dependent manner (Jerkofsky & De Siervo, 1986). VZV lipoproteins may be involved in such changes. VZV gpI is well characterized, but the 5000 and 7000 Mr lipoproteins reported herein have not been reported previously.

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References


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