The herpes simplex virus gene UL26 proteinase in the presence of the UL26.5 gene product promotes the formation of scaffold-like structures

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The herpes simplex virus type 1 (HSV-1) polypeptides encoded by genes UL26 and UL26.5 are thought to form a scaffold around which the capsid shell assembles. The UL26 gene specifies a proteinase that cleaves both itself and the UL26.5 gene product. To study the structure and function of the UL26 and UL26.5 gene products, the proteins were expressed in cells infected with recombinant baculoviruses containing the genes under the control of the polyhedrin promoter. Both polypeptides were made in large amounts, approaching the levels of polyhedrin protein expressed in wild-type baculovirus. The UL26 polypeptide behaved in a similar manner to the protein made in HSV-1-infected cells, cleaving itself rapidly into the capsid proteins VP21 and VP24 and converting the UL26.5 product more slowly into the capsid protein VP22a. The results of immunoblot analysis using antisera specific for the amino-terminal region of the UL26 polypeptide suggested that both the first and second ATGs in the UL26 open reading frame were recognized as translational start signals but the first ATG was the preferred initiation codon as is the case in HSV-1-infected cells. Electron microscopic examination of thin section preparations of cells infected with both the UL26.5- and UL26-recombinant baculoviruses revealed the presence of large numbers of small spherical particles, often arranged in a semi-crystalline array. These clusters of scaffold-like particles were not present in cells infected with UL26-recombinant baculovirus but were observed occasionally in UL26.5-recombinant baculovirus-infected cells. The results suggest that the proteinase, in the absence of other HSV capsid proteins, stimulates the formation of large numbers of scaffold-like particles present either as semi-crystalline arrays or as dispersed structures.

Introduction

The herpes simplex virus type 1 (HSV-1) UL26 gene encodes a proteinase that cleaves the full-length UL26 polypeptide at two positions to generate the capsid proteins, VP21 and VP24 (Liu & Roizman, 1991a; Davison et al., 1992; Preston et al., 1992; Person et al., 1993). The proteinase also cleaves the UL26.5 gene product near its carboxy terminus giving rise to the capsid protein VP22a (Fig. 1). The UL26.5 open reading frame (ORF) overlaps and is in frame with the carboxy-terminal half of the UL26 ORF. The UL26.5 product, however, is not derived from the UL26 polypeptide by proteolytic cleavage but has its own promoter lying within the UL26 ORF (McGeoch et al., 1988; Liu & Roizman, 1991b). Since all the amino acid sequences in the UL26.5 gene product are present in the UL26 polypeptide, the proteinase recognizes the same cleavage site near the carboxy terminus in both proteins. Amino acid sequence analysis of the autoproteolytic cleavage products of a modified UL26 gene expressed in Escherichia coli revealed that the proteinase cleaves between alanine and serine residues at amino acids 247/248 and 610/611 (DiIanni et al., 1993). On the basis of site-specific mutagenesis studies and the effect of various proteinase inhibitors on the UL26 proteinase, it has been proposed that the UL26 product is a novel serine proteinase (Liu & Roizman, 1992, 1993). Both VP24 and the full-length UL26 product are proteolytically active (Liu & Roizman, 1992, 1993; Weinheimer et al., 1993).

Genetic and structural studies suggest that the UL26 and UL26.5 gene products are important in the early stages in the assembly of the virus particle. The capsid protein VP22a, which is the most abundant protein in intermediate (B) capsids, is thought to form a scaffold around which the icosahedral capsid of HSV assembles (Newcomb & Brown, 1989, 1991; Newcomb et al., 1989; Baker et al., 1990). Since VP21 contains all the amino acid sequences present in VP22a, it is likely that this protein is also part of the scaffold, albeit a minor component. VP24, like VP21 and VP22a, is present in the interior of intermediate capsids; however, in contrast to VP22a and VP21, this protein is retained in the capsid.
after DNA encapsidation (Gibson & Roizman, 1972). The precise role of the proteinase is not known. Studies with the HSV-1 temperature-sensitive mutant ts1201, which has a defect in the amino-terminal region of the proteinase, suggest that cleavage of the UL26.5 product and possibly of the proteinase itself alters the morphology of the scaffold and that these changes are essential for the encapsidation of viral DNA (Preston et al., 1983).

To date, every mammalian herpesvirus genome sequenced has genes homologous to UL26 and UL26.5 and in all cases the arrangement of the genes with respect to each other is the same as for the HSV-1 genes (Bacq et al., 1984; Davison & Scott, 1986; Chee et al., 1990; Griffin, 1990; Welch et al., 1991; Telford et al., 1992). Furthermore, detailed studies with human and simian cytomegalovirus proteinases have shown that these enzymes have similar properties to the HSV proteinase which may be active against other herpesviruses makes this enzyme especially attractive as a potential target for antiviral drugs. In this paper we described the high-level expression of the UL26 proteinase and its substrate, UL26.5 polypeptide, in cells infected with recombinant baculoviruses carrying these genes and show that cells infected with both these recombinant viruses produce numerous scaffold-like particles. The production of large amounts of protein should facilitate structural and functional analyses of UL26 and UL26.5 products.

Methods

**Cells.** *Spodoptera frugiperda* (SF) 21-AE cells, derived from SF IPLB-SF-21 cells (Vaughn et al., 1977), were maintained in TC100 medium (Life Technologies) containing 5% fetal calf serum and 100 U/ml penicillin. BHK-21 clone 13 cells (Macpherson & Stoker, 1962) were cultured in Eagle's medium (Life Technologies) containing 10% tryptose phosphate and 10% newborn calf serum.

**Viruses.** The wild-type (wt) HSV-1 used was the non-syncytial form of strain 17 (Brown et al., 1973). The temperature-sensitive mutant ts1201 was isolated from this strain and has a defect in the amino-terminal region of the UL26 proteinase (Preston et al., 1983). Cloned HSV genes were recombined into the baculovirus expression vector AcRFP23lacZ or a derivative of this virus, AcPK6, both of which contain the β-galactosidase gene under the control of the polyhedrin promoter (Kitts et al., 1990; Bishop, 1992). These viruses and wt *Autographa californica* nuclear polyhedrosis virus (AcNPV) were generously supplied by R. D. Possee.

**Construction of recombinant baculovirus transfer vectors.** The HindIII–KpnI fragment from pGX327 and pGX239, containing the UL26 ORF starting from the second ATG codon and the UL26.5 ORF respectively (Preston et al., 1992), were each ligated to pUC18 cleaved with HindIII and KpnI. Both pUC18 recombinant plasmids generated were cleaved with HindIII, incubated with calf intestinal phosphatase, treated with *Escherichia coli* DNA polymerase large fragment in the presence of all four deoxyribonucleotides and ligated to the phosphorylated BglII oligonucleotide linker 5' CAGATCTG 3'. Similarly, the EcoRI site in each of the recombinant plasmids was converted to a BglII site. The complete UL26 ORF was cloned from the plasmid pGX142, containing HSV-1 strain 17 *KpnI* fragment, into pUC19 in two steps. A 663 bp *NlaIII* fragment from pGX142, spanning from the ATG initiation codon of the UL26 gene to just past the *HpaI* site within the gene, was cloned into the *SphI* site in pUC19 (Nicholson, 1992). The *HpaI–KpnI* fragment from pGX142, containing the 3' end of the UL26 gene, was ligated to the pUC19 recombinant plasmid digested with *HpaI* and *KpnI* to generate the complete UL26 gene. BglII sites were created at the *EcoRI* and HindIII sites in this plasmid as described above. The HSV genes flanked by BglII sites were cloned as BglII fragments into the BglII site of the modified baculovirus transfer vector pAcCL29.1 B derived from pAcCL29.1 (Livingstone & Jones, 1989). In this vector the Smal site was converted to a BglII site using the oligonucleotide linker described above.

**Isolation of recombinant baculoviruses.** The recombinant transfer plasmid (2 µg) containing UL26 or the UL26 gene starting from the second ATG (AUL26) was transfected into 10⁶ SF cell together with 0.5 µg Bsu36I-cleaved AcRP23lacZ DNA, using a liposome-mediated procedure essentially as described by Stow (1992) except that a concentration of 10 mg dimethylsulfoxidecetylaminium bromide and 4 mg dioleoyl phosphatidyl ethanolamine in 10 ml H₂O was used in the preparation of liposomes. The transfer vector with the UL26.5 gene was recombined into AcPK6 instead of AcRP23lacZ. The medium was collected from the cells 48 h after transfection and the cell-released virus was titered using the plaque assay of Brown & Faulkner (1977). Virus plaques were stained with neutral red in the presence of 250 µg/ml X-Gal. Viruses producing white plaques were plaque-purified three times and high titre virus stocks prepared. To confirm that each virus contained the correct HSV gene, virus-infected cell DNA was digested with BglII, separated on an agarose gel, blotted on to nitrocellulose and screened for the presence of a novel BglII fragment that hybridized to 32P-labelled cloned HSV-1 *KpnI* fragment, containing both UL26 and UL26.5 sequences and that was the same size as the HSV-1 BglII fragment that was introduced into the baculovirus genome.

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**Fig. 1.** Organization of UL26 and UL26.5 genes and the derivation of the capsid proteins VP21, VP22a and VP24. Numbers refer to amino acid residues in UL26 and the arrows indicate cleavage sites. The number near each cleavage site refers to the alanine residue on the N-terminal side of the scissile bond.
Antisera. Two mouse monoclonal antibodies, MCA 5010 (Rixon et al., 1988) and MCA 406 (Sero tec) which recognize epitopes within VP22a, the cleaved product of UL26.5, were used. In addition four antisera against oligopeptides specific to the UL26 and UL26.5 gene products were raised in rabbits. Polyclonal antisera were raised against oligopeptides specific to UL26 and UL26.5. VP22a, the cleaved product of UL26.5, were used. In addition four antisera against the peptide corresponding to amino acid residues 10 to 21 in the UL26 ORF and raised against the peptide corresponding to the first nine amino acids of the UL26 ORF. Two mouse monoclonal antibodies, MCA 5010 (Rixon et al., 1988) and MCA 406 (Sero tec) which recognize epitopes within VP22a, the cleaved product of UL26.5, were used. In addition four antisera against oligopeptides specific to the UL26 and UL26.5 gene products were raised in rabbits. Polyclonal antisera were raised against oligopeptides specific to the UL26 and UL26.5. VP22a, the cleaved product of UL26.5, were used. In addition four antisera against the peptide corresponding to amino acid residues 10 to 21 in the UL26 ORF and 20995 raised against the peptide corresponding to amino acid residues 10 to 21 in the UL26 ORF and 20999 raised against the peptide corresponding to the first nine amino acids of the UL26 ORF, were produced as described by Preston et al. (1992). To facilitate coupling to the carrier protein β-galactosidase, the two peptides specific for the amino terminus of UL26 polypeptide also contained a tyrosine residue at the amino terminus, and the peptide specific for the carboxy-terminal region of UL26 polypeptide had a tyrosine residue at the carboxy terminus. An antiserum specific for VP21 was raised against the peptide SEKFKMWGAEPVSAPC (amino acid residues 248 to 263 of UL26 ORF) coupled to the carrier protein keyhole limpet haemocyanin via the cysteine residue.

Immunoprecipitation and protein blotting. Immunoprecipitation was carried out essentially as described by Preston et al. (1992) except that Protein A-Sepharose was used instead of Staphylococcus aureus to bind the antibody–antigen complex. The immunoprecipitated polypeptides were analysed by SDS-PAGE using the conditions of Marsden et al. (1978). Cells were fixed and treated with Enhance (Du Pont) prior to being dried. Immunoblotting was performed using the method of Towbin et al. (1979) with some modifications. Briefly, the proteins were separated by SDS-PAGE and transferred on to nitrocellulose membrane. The nitrocellulose membrane was incubated at 37 °C for 30 min in PBS containing 5% BSA, was washed and then incubated overnight at room temperature in PBS containing 0.1% BSA and the primary antibody. The membrane was again washed and incubated for 2 h in PBS containing Protein A conjugated to 20 nm colloidal gold particles (Biocell). After electrophoresis, the gel was stained with Coomassie blue. Lanes 1 and 9, standards; lane 2, AcNPV; lane 3, AcUL26; lane 4, AcUL26; lane 5, AcUL26.5; lane 6, AcPAK6; lane 7, AcRP23lacZ; lane 8, mock-infected Sf cells. The symbols (D, ), (O) indicate HSV polypeptides, β-galactosidase and polyhedrin respectively.

Expression of HSV UL26 and UL26.5 polypeptides

BHK cells were infected with HSV and radiolabelled with [35S]methionine as described by Preston et al. (1992). Sf cells were infected with 10 p.f.u. baculovirus per cell or mock-infected, incubated at room temperature for 1 h, overlaid with TC100 medium containing 5% fetal calf serum and incubated at 28 °C. At 29 h post-infection the medium on the cells was replaced with methionine-free TC100. One hour later, the cells were incubated in fresh methionine-free TC100 containing [35S]methionine for 20 min at 28 °C. The cells were either harvested in radioimmunoprecipitation assay buffer or incubated for various times in TC100 medium containing 5% fetal calf serum and 10 times the normal concentration of methionine before being harvested.

Electron microscopy. Baculovirus-infected cells were prepared for electron microscopy as described by Addison et al. (1984). Immunolocalization was carried out essentially as described by Rixon et al. (1988) except that Epon 812 resin was used instead of Lowicryl K4M and the initial incubation with the primary antibody was for 16 h at room temperature. The UL26.5 gene product was detected using goat anti-mouse IgG coupled to 10 nm colloidal gold particles (Sigma).

Results

Expression of HSV-1 UL26 and UL26.5 polypeptides by recombinant baculoviruses

The HSV-1 genes UL26, ΔUL26 (which encodes a protein lacking the first nine amino acids of UL26 gene product) and UL26.5, under the control of the polyhedrin promoter, were recombined into AcRP23lacZ or AcPAK6, replacing the lacZ gene. Putative recombinant viruses, forming white plaques in the presence of X-gal, were isolated and screened for the ability to produce novel proteins. Viruses that expressed proteins of the expected size for UL26 or UL26.5 gene products and contained the HSV gene were plaque-purified and the virus-induced polypeptides were analysed by SDS-PAGE. Fig. 2 shows mock- and virus-infected cell polypeptides separated on an SDS-containing polyacrylamide gel. The AcUL26-infected cell polypeptide profile contained two major bands, one of M, about 46K containing several polypeptide species and another of M, about 27K (Fig. 2, lane 4). Cells infected with AcΔUL26 also had a major polypeptide of 46K but the lower M, species was slightly smaller than the abundant 27K polypeptide in AcUL26-infected cells (Fig. 2, lane 3). Since the
Characterization of the HSV polypeptides produced in recombinant baculovirus-infected cells

To compare the HSV polypeptides produced in baculovirus-infected cells with the polypeptides present in HSV-1-infected cells, immunoblot analysis was carried out using a variety of antisera specific for sequences within the UL26 or UL26.5 polypeptides. Mock- and virus-infected cell polypeptides, separated by SDS-PAGE, were transferred onto nitrocellulose membranes and incubated with the primary antibody. Antigen-antibody complexes were detected using Protein A coupled to colloidal gold particles and the signal was amplified with a silver enhancing solution. Monoclonal antibody MCA 406, specific for sequences within VP22a, recognized a polypeptide band that comigrated with the uncleaved forms of UL26.5 polypeptide present in wt virus- and ts1201-infected cell profiles, establishing that the UL26.5 polypeptide was expressed in baculovirus-infected cells (Fig. 3a). In the AcUL26- and AcAUL26-infected cell profiles, a slightly higher Mr band of about 46K, which comigrated with a faint band in wt virus-infected cell profile, was detected with the monoclonal antibody. There was no polypeptide band in AcUL26- or AcAUL26-infected cell extracts comigrating with the full-length proteinase present in AcUL26- or AcAUL26-infected cell extracts. When larger amounts of protein were used in the protein blot, a band corresponding to the full-length proteinase was observed in HSV-1 wt virus-infected cell lane but no band of this Mr was detected in AcUL26- or AcAUL26-infected cells (data not shown). To verify that the polypeptide recognized by MCA 406 in AcUL26- and AcAUL26-infected cell extracts corresponded to VP21, a protein blot of mock- and virus-infected cell polypeptides separated by SDS-PAGE was probed with an antiserum raised against an oligopeptide corresponding to sequences in VP21 upstream of UL26.5 amino acid sequences (Fig. 3b). The antibody recognized a polypeptide band of Mr around 46K in AcUL26- and AcAUL26-infected cell polypeptides that was absent.
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from the AcUL26.5-infected cell polypeptide profile. This polypeptide comigrated with a faint band in wt HSV-1-infected cell polypeptide profile, suggesting that this polypeptide was VP21. A similar protein blot was incubated with antiserum raised against an oligopeptide corresponding to the first nine amino acids of UL26 polypeptide and that the first methionine codon in the UL26 ORF was preferentially used. The antiserum failed to react with the abundant low M₇ polypeptide expressed by AcΔUL26 as the protein lacks the first nine amino acids at the amino terminus of the UL26 product. Another antiserum, raised against an oligopeptide corresponding to amino acids 10 to 21 in the UL26 ORF, recognized both the 27K polypeptide in AcUL26-infected cells and the 23K polypeptide in AcΔUL26-infected cells (Fig. 4a). Since the antibody also reacted with a 23K M₇ polypeptide in AcUL26-infected cells, it is likely that there is some initiation of translation from the second methionine in the UL26 ORF. The antibody also detected the full-length proteinase in the ts1201-infected cell polypeptide profile and VP24 in wt HSV-1-infected cells. No band corresponding to the HSV-specific M₇ 23K polypeptide band was observed in the wt HSV-1-infected cells. Because the antibody binds weakly with VP24, it is not clear whether this species is present in HSV-1-infected cells.

The results from the protein blot analysis confirm that the mutant ts1201 displays reduced cleavage of the UL26.5 polypeptide at the non-permissive temperature and also that there was reduced cleavage of the full-length proteinase to VP21 and VP24.

Cleavage of UL26.5 product by the UL26 proteinase in cells infected with AcUL26 and AcUL26.5

Mock- and virus-infected cells were pulse-labelled with [³⁵S]methionine for 20 min and the samples were either harvested immediately or after 2, 4, 6 h or an overnight incubation at 28 °C in non-radioactive medium. Samples were incubated with MCA 5010, specific for sequences...
within VP22a, and the immune precipitates were analysed by SDS-PAGE (Fig. 5). Immune precipitates of extracts from cells co-infected with AcUL26 and AcUL26.5 or infected with AcUL26 alone and pulse-labelled with [35S]methionine contained two minor, high $M_r$ polypeptides of the size expected for full-length UL26 and UL26 cleaved near the carboxy terminus. The samples also contained a major polypeptide of about 46K which comigrated with VP21 in HSV-1-infected cells. These results suggest that cleavage of UL26 to VP21 and VP24 occurs very rapidly. By contrast, cleavage of the UL26.5 polypeptide to VP22a in cells infected with both AcUL26 and AcUL26.5 was slower and complete cleavage was only observed after an overnight incubation as is the case in HSV-1-infected cells (Preston et al., 1983).

To confirm that UL26 expressed by the recombinant baculovirus cleaved the UL26.5 product near its carboxy terminus, immunoblot analysis was carried out on mock- and virus-infected cell polypeptides using antiserum 20999, which recognizes sequences near the carboxy terminus of the full-length UL26.5 polypeptide that are cleaved by the UL26 proteinase, and MCA 406, which is specific for sequences within VP22a (Preston et al., 1992) (Fig. 6). In HSV-1- and AcUL26.5-infected cells and in cells mixedly infected with AcUL26 and AcUL26.5 the
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Fig. 6. Cleavage of UL26.5 gene product by the UL26 proteinase in cells infected with AcUL26 and AcUL26.5 occurs near the carboxy terminus. Protein blot of mock- and virus-infected Sf cells probed with (a) antibody MCA 406, specific for sequences present within VP22a and (b) antibody specific for the carboxy terminus of UL26.5. Samples were prepared as described in Fig. 3. Lane 1, mock-infected Sf cells; lane 2, AcRP231acZ; lane 3, AcPAK6; lane 4, AcUL26-infected cells; lane 5, AcUL26.5-infected cells; lanes 6 and 7, cells mixedly infected with AcUL26 and AcUL26.5; lane 8, wt HSV-1-infected cells; lane 9, mock-infected BHK cells.

antiserum 20999 detected uncleaved forms of the UL26.5 product that comigrated on an SDS–polyacrylamide gel with the higher Mr species of this polypeptide recognized by MCA 406 in the AcUL26.5-infected cell extracts. No major polypeptide species were identified in AcUL26-infected cells using antiserum 20999, supporting the earlier observation that the site near the carboxy terminus of UL26 is rapidly cleaved. The results show that the lower Mr forms of the UL26.5 polypeptide present in cells mixedly-infected with AcUL26 and AcUL26.5 lack sequences near the carboxy terminus and suggest that cleavage was occurring at the carboxy-terminal site.

Electron microscopy of AcUL26- and AcUL26.5-infected cells

When intermediate capsids, which contain large amounts of VP22a, are treated with guanidine–HCl, VP22a is preferentially eluted from the capsids. Upon removal of the denaturant, specific structures form reminiscent of the scaffolds produced by various bacteriophage scaffolding proteins (Newcomb & Brown, 1991). To determine whether the UL26.5 or UL26 gene products could form distinct structures in the absence of any other HSV proteins, electron microscopy was carried out on thin section preparations of insect cells either singly or mixedly infected with AcUL26 and AcUL26.5. Examination of cells infected with AcUL26 revealed the presence of large amounts of fibrous material, mainly localized in the nuclei (Fig. 7a). Similar areas of fibrous material were observed in cells infected with AcUL26.5 although less frequently than in AcUL26-infected cells. In about 1% of AcUL26.5-infected cells analysed, aggregates or areas of dispersed scaffold-like particles 40 to 60 nm in diameter were seen (Fig. 7b). Generally, however, distinctive areas in AcUL26.5-infected cells consisted of fibrous material interspersed with scaffold-like particles (Fig. 7c). In cells mixedly infected with AcUL26 and AcUL26.5, semi-crystalline arrays of scaffold-like particles were frequently observed as well as dispersed particles (Fig. 7d and e). More rarely, fibrous patches or areas intermediate between the fibrous material and arrays of scaffold-like particles were seen. In all cases accumulations of UL26 and UL26.5 polypeptides were generally found in the nucleus, although clusters of HSV protein were also observed in the cytoplasm. In cells infected with AcPAK6, areas of scaffold-like particles or the distinctive fibrous material seen in cells infected with the recombinant baculoviruses were not observed (Fig. 7f). Other fibrous material, however, resulting from aggregation of p10 protein of baculovirus was detected in the nuclei of cells infected with the recombinant or parental baculovirus (van der Wilk et al., 1987).

To confirm that the scaffold-like particles contained UL26.5 polypeptide, immunoelectron microscopy was carried out, using the monoclonal antibody specific for sequences within VP22a (MCA 5010) tagged with goat anti-mouse IgG coupled to colloidal gold (Fig. 8). In
Fig. 7. a-d. For legend see opposite.
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AcUL26 and AcUL26.5 mixedly infected cells gold particles were concentrated over the semi-crystalline arrays of scaffold-like structures, areas of fibrous material and regions intermediate between the fibrous material and clusters of scaffold-like particles, suggesting that the distinctive areas in cells infected by the recombinant viruses resulted from accumulations of HSV protein. Similar results were obtained for the single infections.

Discussion

The HSV-1 genes UL26 and UL26.5 have been expressed in large amounts in insect cells infected with recombinant baculoviruses carrying these genes. Both recombinant viruses grow well in tissue culture, suggesting that production of the foreign protein late in virus infection does not significantly interfere with the replication of the baculovirus. Although HSV UL26 and UL26.5 gene products have also been expressed in bacteria, the proteinase did not appear to be as active as the product from recombinant baculovirus-infected cells (Deckman et al., 1992). The difference could be due simply to the fact that the proteinase in baculovirus-infected cells is at a much higher concentration than the bacterially expressed enzyme. Alternatively, eukaryotic post-translational modifications may be important for enzymatic activity. The observation that in vitro translated proteinase is unable to cleave itself at the N-terminal site supports the idea that some processing of the proteinase may be required for full activity (Liu & Roizman, 1991b; 1992). Deckman et al. (1992) noted that the proteinase expressed in bacteria was processed more rapidly, especially at the N-terminal cleavage site, in the presence of the UL26.5 product than in its absence. Cleavage of the N-terminal site in the proteinase in the absence of UL26.5 product was rapid in the recombinant baculovirus-infected cells. It was observed, however, when cells were co-infected with recombinant viruses expressing UL26 and UL26.5 proteins, that there were slightly greater amounts of the full-length UL26 protein than the UL26 product cleaved at its carboxy-terminal site in comparison with cells singly infected with the recombinant baculovirus expressing the UL26 proteinase.
Fig. 8. Immunoelectron microscopy of thin sections of cells mixedly infected with AcUL26 and AcUL26.5 using antiserum 5010 tagged with goat anti-mouse IgG conjugated to colloidal gold particles. Samples were prepared in the same way as described for Fig. 7. Panels (a) and (b) show a semi-crystalline array of scaffold-like particles; (c) and (d) show fibrous aggregations of HSV protein. Bar markers represent 0.5 μm.
(data not shown). This difference, however, could be explained by the presence of larger amounts of the proteinase in the singly infected cells than in the double infection. Characterization of the UL26 and UL26.5 gene products expressed in cells infected with the recombinant baculoviruses, using a variety of antisera, has shown that these proteins are similar in their properties to the equivalent products synthesized in HSV-infected cells.

Electron microscopic examination of thin sections of AcUL26.5-infected cells revealed that aggregates of UL26.5 product were variable in morphology, ranging from fibrous material to scaffold-like particles, with structures intermediate between these extremes predominating. By contrast, UL26 gene products formed only fibrous material. Since most of the UL26 product in recombinant baculovirus-infected cells is cleaved into VP21 and VP24, it is possible that amino acid sequences at the 5' end of VP21, upstream of the ATG for UL26.5, prevent scaffold formation by VP21. Alternatively, VP24 may have a direct influence on the structure adopted by VP21. VP21, as a consequence of proteolytic cleavage, lacks 25 amino acids present at the carboxy-terminal end of the full-length UL26.5 product. Another possible explanation for the differences in morphology between the UL26.5 and UL26 products is that these 25 amino acids may be required for assembly of the protein into scaffolds. In mixed infections of SF21 cells with AcUL26 and AcUL26.5 semi-crystalline arrays of scaffolds and dispersed scaffolds were frequently observed, whereas in AcUL26.5-infected cells these structures were rarely detected and were usually less prominent in appearance. The scaffolds arising from cells infected with AcUL26.5 were similar in size and morphology to those generated in cells mixedly infected with AcUL26 and AcUL26.5 viruses whereas the internal components of capsids formed in the absence of the UL26 products in cells infected with a panel of recombinant baculoviruses were larger than those formed in the presence of the proteinase (Tatman et al., 1994). The reason for this difference is not clear. It is possible that the capsid shell may influence the morphology of the scaffold.

The results from the analysis of recombinant baculovirus-infected cells suggest that although the UL26.5 gene product has the intrinsic ability to form scaffold-like structures, it does so inefficiently and requires the UL26 gene products for the formation of large numbers of scaffold-like particles. Experiments are in progress to determine whether the VP24 or VP21 component of the proteinase is responsible for stimulating scaffold formation. Interestingly, recent findings from experiments in which cells were infected with recombinant baculoviruses carrying the HSV capsid genes UL18, UL19, UL38 and UL26.5 revealed that capsid formation occurred efficiently in the absence of the UL26 proteinase (Tatman et al., 1994). These findings clearly demonstrate that in the presence of capsid shell proteins UL26 proteinase is not essential for production of large numbers of scaffolds and show that one or more capsid shell proteins can also influence the pathway of assembly of UL26.5 product. The ability of several proteins to enhance scaffold formation may be a mechanism to ensure efficient production of templates for capsid assembly. It is also possible that the influence of capsid shell proteins on scaffold production by the UL26.5 product, which is independent of the proteinase, may be a means of limiting the number of proteinase molecules incorporated into the capsid. It would be interesting to compare the proportion of proteinase molecules in scaffolds formed in the presence or absence of capsid shell proteins. The failure of the UL26 products to form scaffolds in the absence of any other HSV proteins and their inability to participate efficiently in capsid formation in the absence of the UL26.5 product (Tatman et al., 1994) suggest another strategy for limiting the number of copies of the proteinase in capsids.

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