The N Terminus of the Herpes Simplex Virus Type 1 Triplex Protein, VP19C, Cannot Be Detected on the Surface of the Capsid Shell by Using an Antibody (Hemagglutinin) Epitope Tag

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The herpes simplex virus (HSV) triplex is a complex of three protein subunits, VP19C and a dimer of VP23 that is essential for capsid assembly. We have derived HSV-1 recombinant viruses that contain monomeric red fluorescent protein (mRFP1), a Flu hemagglutinin (HA) epitope, and a six-histidine tag fused to the amino terminus of VP19C. These viruses were capable of growth on Vero cells, indicating that the amino terminus of VP19C could tolerate these fusions. By use of immunoelectron microscopy methods, capsids that express VP19C-mRFP but not VP19C-HA were labeled with gold particles when incubated with the corresponding antibody. Our conclusion from this data is that a large tag at the N terminus of VP19C was sufficiently exposed on the capsid surface for polyclonal antibody reactivity, while the small HA epitope was inaccessible to the antibody. These data indicate that an epitope tag at the amino terminus of VP19C is not exposed at the capsid surface for reactivity to its antibody.
and a virus designated K19C-Spe1 was isolated on Vero cells and plaque purified further.

The growth properties of the recombinant viruses were examined by infecting Vero and C32 cells and determining virus yields at different times postinfection (Fig. 1). K19C-Spe1, K19C-HA, and K19C-HIS gave rise to virus yields that were comparable to those of the wild-type virus, KOS, at 24 h postinfection (Fig. 1). The growth of K19C-mRFP was reduced 14-fold relative to that of the wild-type virus (Fig. 1). The growth of K19C-mRFP was recovered partially (sevenfold) by replication in the complementing cell line C32 (Fig. 1). The VP19C trans complementation in C32 cells was never at the level seen for wild-type virus (data not shown). The growth of K19C-Spe1 was comparable to that of wild-type virus, indicating that the Spe1 site did not alter virus replication (Fig. 1).

To confirm the expression of the tagged VP19C polypeptides, infected Vero cell lysates were analyzed at different times postinfection using antibodies to the different tags in Western blot assays (Fig. 2). In the case of antibodies to HA, six-histidine, and DsRed, a polypeptide with the correct mobility was detected in extracts derived from cells infected with the respective virus (Fig. 2). The DsRed antibody also recognizes mRFP protein. There was little reactivity of the antibodies to either the wild-type protein or mock-infected cell proteins. There was a corresponding mobility shift in the VP19C-mRFP polypeptide due to the fusion of the mRFP (25 kDa) to VP19C (50 kDa). VP19C-HA and VP19C-HIS polypeptides also exhibited slightly slower mobilities relative to that of the wild-type protein (Fig. 2). Since VP19C is expressed from a late gene, there is very little protein accumulation at 8 h postinfection. The highly reactive HA antibody was able to detect low amounts of this protein at early times (Fig. 2, αHA panel).

[35S]methionine-radiolabeled lysates were sedimented through sucrose gradients in order to isolate and analyze in-
tranuclear capsids (9, 17). The polypeptide composition of the capsids was examined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Fig. 3). The mature C capsid fractions for all the viruses were examined. The capsids isolated all contained the capsid shell proteins; the only difference between the different capsids was the lower mobility of the tagged VP19C polypeptides, which was more evident for the VP19C-mRFP polypeptide (Fig. 3, filled circle). The levels of C capsids derived from K19C-mRFP-infected cell lysates were much lower than the levels of C capsids derived from infected cell lysates of the other viruses (Fig. 3).

In order to determine the surface topology of the different tags fused to VP19C, we carried out immuno-EM for purified capsids using antibodies to the various tags (Fig. 4). B capsids were purified after sedimentation of infected cell lysates through sucrose gradients. Capsids were adsorbed by placing a drop on freshly ionized carbon- and Formvar-coated nickel grids for 5 min, washed eight times by floating grids on a series of drops of deionized H2O, and blocked for 1 h on a drop of 10% fetal calf serum in phosphate-buffered saline. Grids were then floated on a 10-μl drop of either rabbit anti-DsRed (mRFP), 10 μg/ml (Chemicon AB3216), mouse anti-HA (33 μg/ml; Covance 12CA5), or mouse anti-VP5 (diluted 1:20; LP12) and incubated at 4°C overnight. Grids were washed eight times in a wash buffer (1% fetal calf serum in phosphate-buffered saline) and floated on 10-μl donkey anti-rabbit 12-nm Au conjugate (diluted 1:30) or donkey anti-mouse 12-nm Au conjugate (diluted 1:40) (Jackson Research Laboratories) for 2 h at room temperature, washed eight times with wash buffer and five times with deionized H2O, briefly floated on a drop of 2% uranyl acetate, partially dried by touching the side of the grid to filter paper, allowed to air dry, and observed with a
The data were analyzed with Microsoft Excel’s test function. Numerous gold particles associated with KOS capsids (Fig. 4E) were evident when VP5 antibodies (LP12) were used. None or very few gold particles were bound to KOS capsids when HA (Fig. 4C) or DsRed (Fig. 4A) antibodies were used. K19C-HA capsids exhibited little or no labeling when the capsids were reacted with HA antiserum (Fig. 4D, arrowhead). This was not due to the loss of the epitope, because with similar sucrose gradient fractions, a 50-kDa polypeptide reacted with HA antiserum in Western blots (data not shown). Many gold particles were observed bound to K19C-mRFP B capsids when DsRed antibody was used (Fig. 4B). A quantitative analysis was performed for these capsids as well as for KOS B capsids reacted with DsRed antibody. Intact capsids were enumerated, and the number of gold particles bound to the capsids was determined. The data revealed that KOS capsids exhibited little or no labeling (KOS, 0.27 gold particles/capsid) but that K19C-mRFP capsids exhibited significant labeling compared to KOS capsids (K19C-mRFP, 4.41 gold particles/capsid; r = 9.12; P value, 2.85 × 10^{-17}; n = 41 [one-tailed r test]). The mRFP tag was the only one that reacted with its cognate antibody, as judged by gold binding. We also generated a virus in which the HA tag was followed by a small peptide spacer sequence [Pro (Ser, Ala)n Pro] prior to the VP19C sequence. It was thought that this spacer sequence may expose the HA sequence at the capsid shell surface. B capsids isolated from this virus similarly did not react with antibodies to HA (data not shown).

Because many of our studies have generally focused on studying bimolecular protein-protein interactions in isolated systems, our goal was to probe a multiprotein complex to ascertain the topology of protein domains. Hence, our aim was to determine the location of the N terminus of VP19C on the capsid shell. Use of this method of residue-specific ligand attachment to locate a protein domain in conjunction with high-resolution cryo-EM could potentially yield a three-dimensional map of the structural motif in the capsid shell. This method has been used successfully to locate the protein domains in the capsids of viruses (3, 12, 14, 18). However, by using immuno-EM methods, it was not possible to detect the tag in the assembled capsid, indicating that the HA epitope was not exposed on the surface of the capsid shell. As judged by immuno-EM methods.

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REFERENCES