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Processing of the Herpes Simplex Virus Type 2 Glycoprotein gG-2 Results in Secretion of a 34,000-M_r Cleavage Product

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Herpes simplex virus type 2 glycoprotein gG-2 undergoes a cleavage event during its synthesis and processing. The focus of this report is on the detection and fate of the small-molecular-weight component of gG-2, designated the 34K component. In cultures containing the inhibitor monensin, a 31K component accumulated within infected cells. In contrast, the intracellular accumulation of this 31K precursor was not detected in cultures grown in the absence of the inhibitor. However, the 34K component of gG-2 was found in the extracellular culture fluid. The data suggest that the 31K high-mannose cleavage product of gG-2 is further glycosylated and rapidly secreted from herpes simplex virus type 2-infected cells; however, if glycosylation is perturbed, the 31K high-mannose form remains cell associated.

Herpes simplex virus type 2 (HSV-2) encodes several antigenically distinct virus-specific glycoproteins, including gB-2, gC-2, gD-2, gE-2, and gG-2. The synthesis of glycoproteins gB, gC, gD, and gE has been previously described (see the review by Spear [7]). The pathway of the synthesis of gG-2 appears to involve the cleavage of a gG-2 precursor followed by further glycosylation of one of the cleavage products to form the mature gG-2 glycoprotein (1). The gG-2 glycoprotein is apparently synthesized as a cotranslationally glycosylated high-mannose intermediate with an apparent molecular weight of 104,000 (104K intermediate). This 104K intermediate is subsequently cleaved to generate 31,000- and 72,000-dalton high-mannose intermediates (31K and 72K intermediates, respectively), with the 72K high-mannose intermediate being further glycosylated to the mature gG-2 glycoprotein of 108,000 daltons (manuscript in preparation). The detection and fate of the 31K high-mannose cleavage product is the focus of this report.

Studies on the synthesis of the 31K high-mannose intermediate incorporated the use of an antiserum reactive with this 31K component. This monospecific antiserum (designated anti-pgG-2) was prepared to the purified 104K precursor by previously described methods (3). The reactivities of the anti-pgG-2 antiserum in immunoblotting are shown in Fig. 1. In this experiment, HEp-2 cells were infected with strain 186 of HSV-2 by previously described procedures (5). After 1 h of virus adsorption, the cultures were either harvested or maintained in the presence of 10^{-6} M monensin (an ionophore that disrupts the normal transport and processing functions of the Golgi apparatus [8]) until harvest at 16 h postinfection. Whole cell lysates were solubilized and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting (2) with anti-pgG-2 serum or anti-gG-2 serum (prepared to the mature gG-2 glycoprotein). Lanes containing samples harvested at 1 h postinfection show material from the input inoculum detected by the two antisera (Fig. 1). In cultures maintained in the presence of monensin, the 104K precursor as well as the 72K and 31K cleavage products were detected by the anti-pgG-2 antiserum. The anti-gG-2 antiserum was also reactive with the 104K precursor and the 72K cleavage product; however, it was not reactive with the 31K cleavage product. A diffuse glycoprotein band of 96,000 daltons was also detected by the anti-gG-2 antiserum. This glycoprotein component may represent partially processed gG-2 that accumulates in the presence of monensin and is currently under further study. The anti-pG-2 antiserum was used for the subsequent experiments described in this report.

Parallel cultures of HEp-2 cells were infected with strain 186 of HSV-2 at a multiplicity of infection of approximately 50 PFU. After 1 h of adsorption, the cultures were maintained in the presence or absence of 10^{-6} M monensin to promote the accumulation of the high-mannose precursors (9). At 10 h postinfection, cycloheximide (final concentration, 50 μg/ml), was added to the cultures to follow the processing of precursor(s) to final product in the absence of further protein synthesis. Cultures were then harvested at different times following cycloheximide addition, and the solubilized cell extracts were analyzed by SDS-PAGE and immunoblotting with anti-pgG-2 antiserum. The level of the 104K component was observed to diminish at later times after the inhibition of protein synthesis caused by cycloheximide addition (Fig. 2). Concomitantly, a significant increase in the level of the 31K component was observed. This result supports the concept that the 31K component detected by anti-pgG-2 antiserum represents the cleavage product of the 104K high-mannose intermediate.

The kinetics of appearance of the 31K high-mannose component synthesized in the presence or absence of monensin were determined. HSV-2-infected HEp-2 cells were cultured in the presence or absence of the inhibitor. Whole cell lysates were harvested at various times after infection and then analyzed by SDS-PAGE and immunoblotting with anti-pgG-2 antiserum (Fig. 3). In infected cells maintained in the absence of monensin, the 72K and 104K high-mannose intermediates were readily detected, whereas detectable levels of the 31K protein was not observed. However, two proteins migrating just above the 31K protein position were detectable in small amounts and may represent processed forms of the 31K component that remain cell associated (see below). In contrast, cells cultured in the

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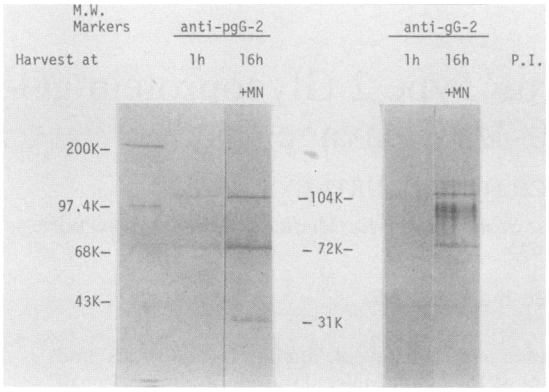

FIG. 1. Reaction of the anti-pgG-2 antiserum with the 31K component. HSV-2-infected HEp-2 cell cultures maintained in the presence of monensin (+MN) were harvested either following the 1-h virus adsorption period or at 16 h postinfection (P.I.). Harvested cell cultures were solubilized and analyzed by immunoblotting with either anti-pgG-2 or anti-gG-2 antiserum. M.W., Molecular weight.

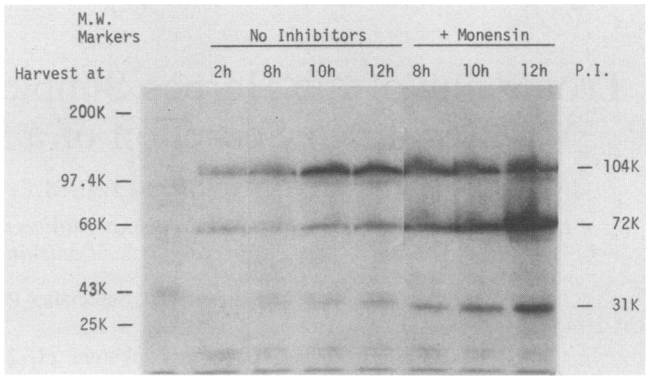

FIG. 3. Intracellular synthesis of the 31K high-mannose product in the presence or absence of monensin. HSV-2-infected HEp-2 cells were cultured in the presence or absence of monensin, and at the indicated times post-infection (P.I.), cultures were harvested. Solubilized cell extracts were then analyzed by SDS-PAGE and immunoblotting with anti-pgG-2 serum. The profile shown in the lane designated 2 h demonstrates antigenic reactivity resulting from gG-2-specific material associated with the input inoculum. M.W., Molecular weight.

presence of monensin contained significant levels of the 31K component as well as the 72K and 104K components. These results suggest that the 31K cleavage product is not present in detectable levels within infected cells maintained in the absence of monensin. However, when the processing events associated with the Golgi apparatus are perturbed, an apparent accumulation of the 31K component occurs.

To determine the fate of the 31K component in HSV-2-infected cells maintained in the absence of any inhibitor, we examined the extracellular medium from infected cells. Media from HSV-2-infected HEp-2 cultures maintained in the presence or absence of monensin were harvested and centrifuged at 13,000 × *g* for 30 min at 4°C to pellet infected-cell debris. The clarified supernatant obtained was then precipitated with trichloroacetic acid at a final concentration of 6% (wt/vol). The precipitate was solubilized in 1 M Tris (pH 6.6) and further precipitated in acetone at −20°C overnight. The precipitated proteins were then analyzed by SDS-PAGE and immunoblotting with anti-pgG-2 antiserum (Fig. 4). A protein of 34,000 daltons (34K protein) was detected in the extracellular medium from cells cultured in the absence of monensin at 8 h postinfection, and increasing levels were observed at 10 and 12 h postinfection. A similar protein was not detected in the extracellular medium from cells cultured in the presence of monensin.

These results suggest that if the normal processing function of the Golgi apparatus is disrupted, the further glycosylation of the 31K component of gG-2 is inhibited, and the 31K component remains cell associated. In contrast, in the absence of any inhibitor, the 31K component is further processed to a 34K component, which is apparently secreted

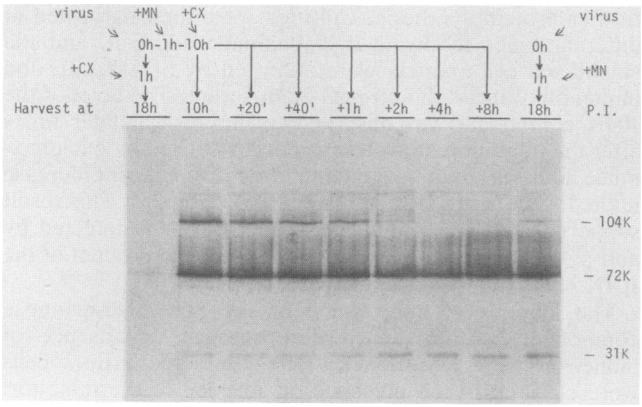

FIG. 2. Processing of the 104K high-mannose intermediate to the 72K and 31K high-mannose intermediates following inhibition of further protein synthesis. Parallel cultures of HEp-2 cells were infected with HSV-2 strain 186, and following a 1-h virus adsorption period, the cultures were maintained in the presence of monensin (+MN). At 10 h postinfection, cycloheximide (+CX) was added and maintained in these cultures until harvesting at the indicated times. Control cultures (containing monensin) were maintained in the presence or absence of cycloheximide from 1 to 18 h postinfection (P.I.). The solubilized cell extracts were analyzed by SDS-PAGE and immunoblotting with anti-pgG-2 serum.

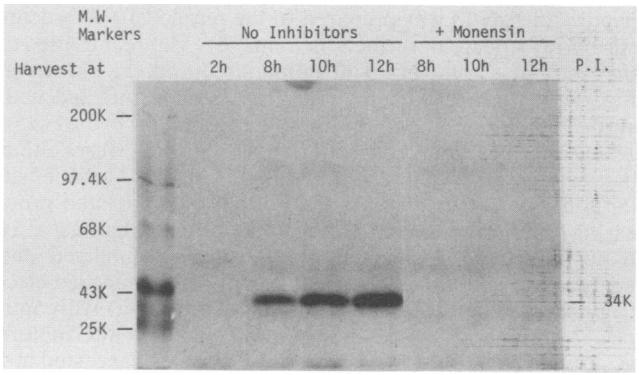

FIG. 4. Extracellular secretion of a 34K product in the presence or absence of monensin. Culture medium from cultures grown in the presence or absence of monensin as described in the legend to Fig. 3 was harvested at various times postinfection (P.I.) and centrifuged for 30 min at 13,000 × *g* at 4°C. Proteins were precipitated from the clarified culture fluids by the addition of trichloroacetic acid at a final concentration of 6% (wt/vol). The precipitated proteins were then analyzed by SDS-PAGE and immunoblotting with anti-pgG-2 serum. M.W., Molecular weight.

from infected cells. This event results in the 34K component being readily detectable in the extracellular medium.

The observation that the 34K cleavage product is secreted during the maturation of glycoprotein gG-2 represents the first known example of such an event associated with the synthesis of a herpes simplex virus glycoprotein. In the synthesis of gX, a suggested homolog of gG-2 in pseudorabies virus (4), a cleavage, processing, and secretion event has also been suggested (6). The role of the 34K secreted product in the infectious cycle of the virus is unknown. However, the potential role that such a secreted virus-specific glycoprotein may play in the modulation of the immune system of the host to virus infection deserves further study.

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