A Study of the Glycoproteins of *Autographa californica* Nuclear Polyhedrosis Virus (AcNPV)

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Pulse labeling with tritiated mannose was used to follow the time course of *Autographa californica* nuclear polyhedrosis virus (AcNPV) glycoprotein synthesis in *Spodoptera frugiperda* IPLB-21 cells. Nine viral-induced intracellular glycoproteins were first detected from as early as 2 hr postinoculation (67K, early phase) to as late as 14 hr (36K and 19K glycoproteins, intermediate phase). Glycosylation of these proteins was observed to continue to the end of the experiment (28 hr postinoculation). Seven of these intracellular glycoproteins could also be detected in infected *Trichoplusia ni* TN-368 cells 24 hr postinoculation. When the glycosylation inhibitor tunicamycin was present (from 0 hr postinoculation) there was no detectable glycosylation of any of these viral-induced glycoproteins. Metabolic labeling of the nonoccluded virus budded from IPLB-21 and TN-368 with tritiated mannose or *N*-acetylglucosamine identified 11 structural glycoproteins, 8 of which were identical in both virus preparations. All of these structural glycoproteins were sensitive to the inhibitory action of tunicamycin. A single 42K structural glycoprotein was detected (with acetylglucosamine only) in the occluded form of AcNPV. Glycosylation of this structural protein appeared to be insensitive to tunicamycin. Lactoperoxidase-catalyzed radioiodination was used to determine which of the virus structural glycoproteins are exposed on the virion surface.

INTRODUCTION

The occurrence of glycoproteins as structural components of enveloped viruses may well be universal (Patzer *et al.*, 1979). A variety of functions have been suggested for viral glycoproteins such as virus envelope acquisition (Payne and Kristensson, 1982; Simons and Garoff, 1980), virus attachment (Bächi *et al.*, 1977), penetration (Little *et al.*, 1981; Huang *et al.*, 1980), and uncoating (Lenard and Miller, 1982). Recently, we demonstrated that the glycoproteins of the nonoccluded form of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) are necessary for the infection of *Trichoplusia ni* cells but not for the release of enveloped progeny virus (Stiles *et al.*, 1983a). In spite of the importance of viral glycoproteins, little work

has been done to characterize the glycoproteins of the insect pathogenic baculoviruses. To date, only four studies have appeared, three involving the structural glycoproteins of AcNPV (Maruniak, 1979; Dobos and Cochran, 1980; Goldstein and McIntosh, 1980) and the fourth dealing with the structural glycoproteins of the nonoccluded baculovirus, Hz-1 (Burand *et al.*, 1983). The time sequence of intracellular viral-specific glycoprotein synthesis has not yet been determined for any baculovirus.

To gain a better understanding of the role of baculovirus glycoproteins we have used the 1A clone of AcNPV (Wood, 1980b) to follow the time course of intracellular viral-induced glycoprotein synthesis in cell culture, to compare the intracellular viral glycoproteins to structural viral glycoproteins, and to determine which structural proteins are exposed on the surface of the virions. Comparisons were also made be-

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tween the viral glycoproteins of AcNPV grown in two different cell lines (*Spodoptera frugiperda* IPLB-21 and *Trichoplusia ni* TN-368). The glycosylation inhibitor tunicamycin was used to ensure that incorporated label from tritiated sugars was present in glycoproteins. This antibiotic is known to specifically inhibit the *N*-glycosidic glycosylation of proteins in animal cells (Hubbard and Ivatt, 1981; Takatsuki and Tamura, 1982) and insect cells (Butters *et al.*, 1981).

MATERIALS AND METHODS

Cells and virus. *Trichoplusia ni* (TN-368) and *Spodoptera frugiperda* (IPLB-21) cells were maintained with TNMFH medium (Hink, 1970) modified according to Wood (1980a). The plaque-purified 1A clone of AcNPV (Wood, 1980b) was used throughout these experiments.

Intracellular viral glycoproteins. TN-368 or IPLB-21 cells (3×10^5 cells) were seeded into each well of a Falcon Multiwell Plate and inoculated with 3×10^6 plaque-forming units of 1A virus (m.o.i. = 10) by centrifugation at 1000 g for 1 hr (Wood, 1977). The virus inoculum was removed and replaced either with medium or medium containing 10 µg/ml tunicamycin (TM) and incubated at 26° (Stiles *et al.*, 1983a). At appropriate times postinoculation (pi) cells in two wells were washed and then starved for 2 hr in low sugar medium (modified TNMFH medium without sucrose or fucose, 1/10 the normal amount of glucose, with dialyzed fetal calf serum added to a final 10% (v/v) concentration and sorbitol added to adjust the osmotic concentration) plus 10 µg/ml TM when appropriate. After starving, 40 µCi (final concentration 130 µCi/ml) of [2-³H]mannose (New England Nuclear Corp.), *N*-[1-³H]acetylglucosamine (Amersham/Searle) or [6-³H]fucose (Amersham/Searle) was added to each well. After a 2-hr labeling period the cells were removed, washed once with Graces medium minus amino acids and pelleted at 300 g for 2 min. The cells were then suspended in 50 µl of distilled water, sonicated at 50 W for 4–5 sec (Heat Systems, model W185 cell disruptor) and 5-µl ali-

quots removed for a modified Lowry protein assay (Markwell *et al.*, 1981). The remaining cell sample was combined with an equal volume of 2X Laemmli buffer (1970) and boiled 2–3 min.

Preparation and purification of ³H-labeled AcNPV. TN-368 or IPLB-21 cells were seeded into tissue culture flasks (1×10^6 cells/cm² surface area) and allowed 1 hr for attachment. The medium was removed and a small volume of fresh medium containing 1A AcNPV (m.o.i. = 10) was added to each flask. The flasks were rocked gently for 1 hr (25°) and then additional medium was added (half the flasks received medium plus 10 µg/ml TM). At 12 hr pi the medium and virus inoculum was removed and 5 ml (for a 75-cm² flask) of low sugar medium (or low sugar medium plus TM) were added. The cells were starved for 2 hr after which 250 µCi of [³H]mannose ([³H]man), or *N*-[H³]acetylglucosamine ([³H]GlcNAc) was added to each flask. At 24 hr pi 10 ml of complete medium (or medium plus TM) was added to each flask.

At 48 hr pi the medium was carefully removed and fresh medium (or medium plus TM) added to the flasks. The medium containing the budded, nonoccluded virus (NOV) was given a low speed centrifugation (300 g, 3 min) to remove cells and then layered over 20% (w/w) sucrose (1 mM Tris buffer, pH 7.5) and centrifuged for 1 hr at 66,000 g and 5°. The virus pellet was suspended in 1 mM Tris buffer (pH 7.5) and then layered onto a 25–55% w/w sucrose (1 mM Tris buffer, pH 7.5) gradient and centrifuged for 3 hr at 80,000 g and 5°. The virus band was collected, diluted with 3 vol 1 mM Tris buffer (pH 7.5), and centrifuged 1 hr at 66,000 g, 5°. The virus pellet was suspended in 50 µl of 1 mM Tris buffer (pH 7.5) and a 5-µl aliquot removed for an optical density determination at 260 nm. The remaining virus samples were mixed with an equal volume of 2X Laemmli buffer and boiled 2–3 min.

At 4–5 days pi the infected cells were harvested from the flasks and the occlusion bodies purified using the SDS–NaCl procedure of Wood (1980b). Purified occlusion bodies (OBs) were dissolved for 15 min in 50 mM Na₂CO₃ at room temperature and

then mixed with an equal volume of 100 mM Tris buffer, pH 7.5, and centrifuged for 15 min at low speed (1000 g) to pellet undissolved OBs. The supernatant was then layered over 25% (w/w) sucrose (1 mM Tris buffer, pH 8.5) and centrifuged at 66,000 g and 5° for 1 hr. The virus pellet was suspended in 50 μl of 1 mM Tris buffer (pH 7.5) and a 5-μl aliquot removed to determine the optical density at 260 nm. The remaining virus samples were mixed with an equal volume of 2X Laemmli buffer and boiled 2–3 min.

Radioiodination of virus envelope surface proteins. Unlabeled NOV was purified as described above. Unlabeled occluded virus released from occlusion bodies (approximately 1 × 10^9) was further purified by banding on a 25–65% (w/w) sucrose gradient (1 mM Tris buffer, pH 7.5) at 80,000 g and 5° (3 hr). Both the NOV and occluded virus bands were collected from the sucrose gradients immediately before iodination. The purified virus (in 1–2 ml of sucrose)

was mixed with 0.2 ml of 100 mM Tris buffer, pH 7.5. The following were then added in order: 75 μl of 10% glucose, 11 mU of glucose oxidase (Sigma), 11 mU of lactoperoxidase (Calbiochem), and 150 μCi of carrier free Na^{125}I (Amersham/Searle) (modification of Hubbard and Cohn, 1972). The mixture was incubated at room temperature for 45 min and then diluted with 2 vol of 10 mM Tris buffer (pH 7.5) to stop the reaction. The labeled virus was immediately layered over a cushion of sucrose (20% w/w for NOV, 35% w/w for occluded virus) in 1 mM Tris buffer, pH 7.5 and centrifuged 1 hr at 60,000 g, 5°. The virus pellet was suspended in 100 μl of Laemmli (1970) buffer and boiled 2–3 min.

Acid hydrolysis of ^3H-labeled virus structural glycoproteins. To check for interconversion of the labeled sugars to other sugars before being incorporated into virus glycoproteins, NOV produced in TN-368 cells and labeled with [^3H]man or [^3H]-GlcNAc was purified as described above.
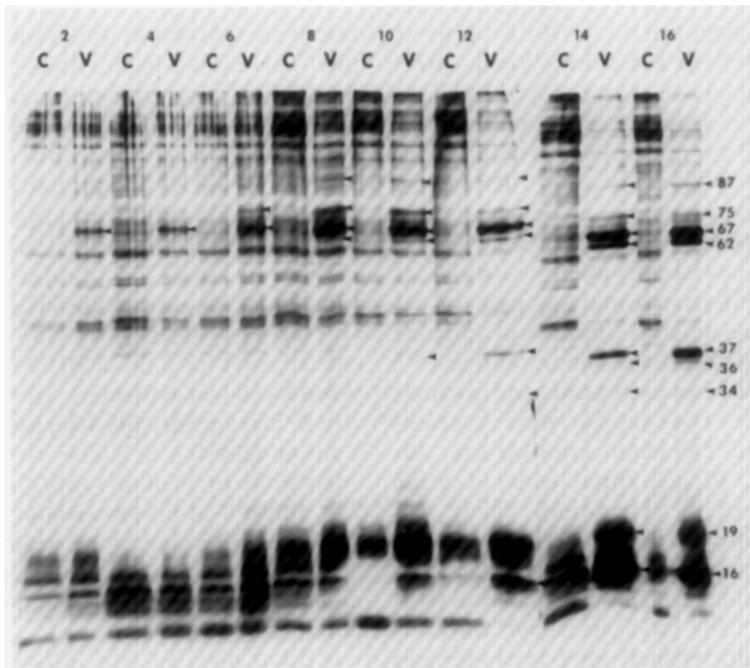
FIG. 1. Time course of AcNPV viral-induced intracellular glycoproteins in IPLB-21 cells labeled with [^3H]mannose. The sample times in hours postinoculation are shown at the top of the figure and all lanes contain the same total amount of TCA-precipitable tritium counts. C, uninfected control cells; V, virus-infected cells. Arrows indicate viral-induced glycoproteins and their approximate molecular weights (×10^{-3}) are given.

The virus (in water) was combined with an equal volume of 20% cold TCA and kept on ice overnight. The mixture was then centrifuged at 1000 g for 15 min, the supernatant poured off and the pellet suspended in 5 ml of chloroform–methanol (2:1) to extract the glycolipids. The extraction was carried out overnight at 5°. The proteins were then pelleted at 1000 g for 15 min and the supernatant discarded. Acid hydrolysis of the samples and thin layer chromatography were carried out according to Klenk et al. (1970). The pellets were dried under a stream of N_2 gas and 1 N HCl added. The mixture was then sealed under N_2 and heated at 100° for 12 hr. The residue was dissolved twice in methanol and dried under N_2 to remove all the acid. The residue was then dissolved in 50% methanol and spotted side by side with unlabeled sugar standards (in 50% methanol) onto a precoated cellulose TLC plastic sheet (Merck), which was divided into lanes 2-cm wide. The solvent system was ethyl acetate–pyridine–acetic acid–water (5:5:1:3) and the chromatogram was developed twice. The marker sugars (glucose, fucose, galactose, mannose, glucosamine, and galactosamine) were stained with an alkaline silver nitrate solution (Krebs et al., 1969) and photographed immediately after development of the spots. The virus sample lanes were cut horizontally into 3-mm wide strips and counted in Biofluor cocktail (New England Nuclear Corp.).

Polyacrylamide gel electrophoresis, autoradiography, and fluorography. Virus samples were dissociated and applied to discontinuous sodium dodecyl sulfate-polyacrylamide gels according to Laemmli (1970). Aliquots of the virus samples were TCA-precipitated to determine the amount of label incorporated. Gels 15 × 15 × 0.15 cm with a 4% stacking and 11% running gels were electrophoresed at a constant 6 W per gel. Gels with ^{125}I-labeled proteins were dried immediately onto No. 1 Whatman filter paper and exposed to a sheet of Kodak X-Omat X-ray film at −70°. ^{3}H-labeled gels were first treated with En[3]Hance (New England Nuclear Corp.) before drying and exposure to X-ray film at −70°.

Molecular weights of labeled proteins were determined by comparison with the ^{14}C-labeled protein standards (Amersham/Searle) phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, and lysozyme.

RESULTS

Intracellular viral-induced glycoproteins. Following infection of IPLB-21 cells and pulse labeling with [^{3}H]man, the synthesis
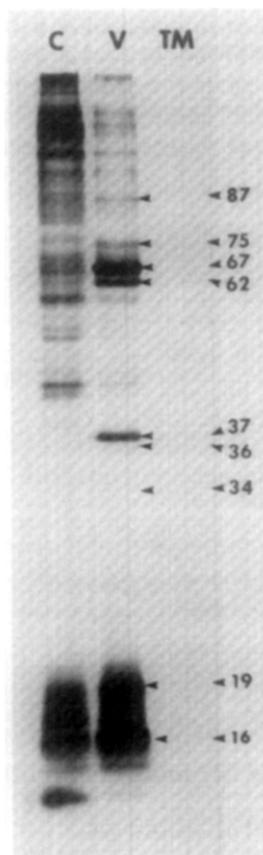
FIG. 2. Labeling of AcNPV intracellular glycoproteins with [^{3}H]mannose with Tunicamycin control. C, uninfected control IPLB-21 cells; V, infected IPLB-21 cells 14 hr pi; TM, tunicamycin treated infected IPLB-21 cells 14 hr pi (note the absence of label incorporation when 10 μg/ml TM was added to cells at 0 hr pi). V and TM lanes contain the same amount of total protein as determined by a modified Lowry (Markwell et al., 1981). Approximate molecular weights (×10⁻³) are indicated and arrows mark the glycoproteins.

of nine viral-induced glycoproteins was observed (Fig. 1). The glycoproteins were detected as early as 2 hr pi (67K) to as late as 14 hr pi (36K and 19K glycoproteins). Glycosylation of these proteins continued through to the end of the experiment (28 hr pi). Pulse labeling with [³H]GlcNAc at 24 hr pi gave identical results as the [³H]man labeling pattern.

To ascertain that the identified proteins were glycosylated and not labeled with metabolically altered forms of the labeled compounds, tunicamycin (TM) was used as a control. As shown in Fig. 2, continuous incubation of infected cells with TM from 0 hr pi followed by pulse labeling with [³H]man at 14 hr pi resulted in undetectable levels of label incorporation. Label incorporation was detected in the presence of tunicamycin prior to 6 hr pi indicating that TM requires approximately 7 hr to enter the cell in sufficient quantity to completely inhibit glycosylation (data not shown).

Incorporation of these labeled sugars was low at early time periods in infected TN-368 cells (data not shown). However, pulse labeling at 24 hr pi with [³H]man and [³H]GlcNAc detected 7 viral-induced glycoproteins (Fig. 3). Labeling of the 87K and 34K proteins observed in IPLB-21 cells was not detected.

Structural glycoproteins of the nonoccluded virus (NOV). The pattern of viral glycoproteins (vpg) for NOV produced from IPLB-21 and TN-368 cells is shown in Fig. 4. NOV from IPLB-21 and TN-368 cells consistently exhibited a total of eleven glycoproteins. The IPLB-21 NOV, however, had three viral glycoproteins (vpg 37, vpg 36, and vpg 34) not seen in TN-368 NOV, while the TN-368 NOV also had three unique viral glycoproteins (vpg 100, vpg 41, and vpg 16). NOV produced from IPLB-21 or TN-368 cells treated with 10 μg/ml tunicamycin had no detectable incorporation of ³H-labeled sugars.

An effort was made to determine the approximate amount of interconversion of the labeled sugar to other sugars before their incorporation into the NOV glycoproteins. When samples of NOV from IPLB-21 cells labeled with [³H]man or [³H]GlcNAc were hydrolyzed with acid and
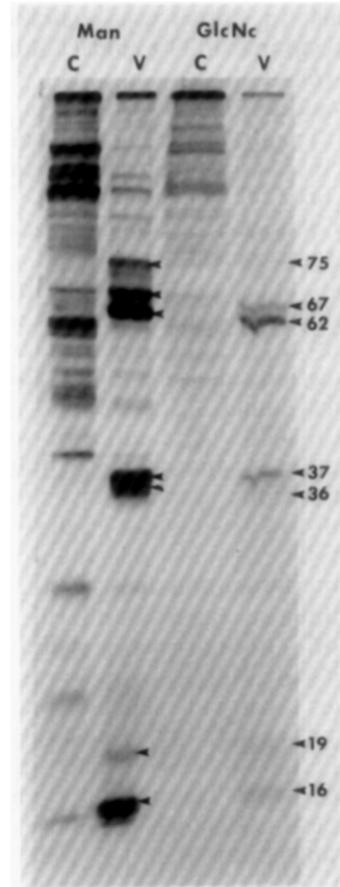
FIG. 3. AcNPV viral-induced intracellular glycoproteins in TN-368 cells at 24 hr pi labeled with [³H]mannose (Man) or N-[³H]acetylglucosamine (GlcNAc). C, uninfected control cells; V, virus-infected cells. Approximate molecular weights (×10⁻³) are indicated and arrows mark the glycoproteins.

the liberated sugars separated by thin layer chromotography it was observed that both sugars underwent some interconversion (Fig. 5). The acid hydrolysis procedure used cleaves off acetyl groups on the sugars so in Fig. 5 the [³H]GlcNAc label shows up as glucosamine with a small amount of conversion (perhaps 10%) to galactosamine (represented by the slight shoulder on the peak). It was found that approximately 20% of the ³H from mannose was incorporated as fucose as shown by the twin peaks in Fig. 5. It is also noteworthy that relatively little label was observed between the origin and the sugar peaks as this is
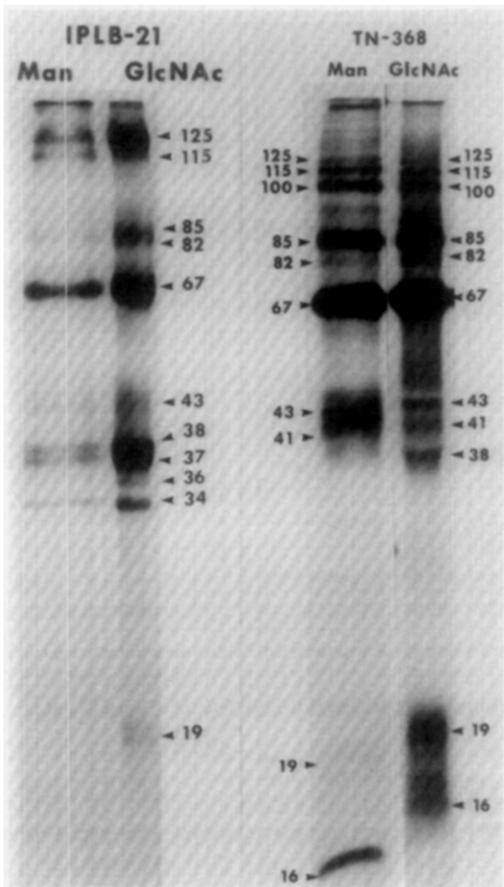
FIG. 4. A comparison of the structural glycoproteins of AcNPV–NOV derived from IPLB-21 and TN-368 cells. The virus was harvested at 48 hr pi and each well was loaded with approximately the same total number of virus particles. Man, [³H]mannose label; GlcNAc, N-[³H]acetylglucosamine label. The approximate molecular weights (×10⁻³) of the glycoproteins are indicated and arrows mark the glycoproteins.

the region where the liberated amino acids are found (Klenk et al., 1970).

Occluded virus glycoproteins. Under the conditions used here the IPLB-21 cells produced few virus OBs; therefore, only OBs from TN-368 cells were examined for glycoproteins. The occluded virus was found to contain a single 42K structural glycoprotein which labeled with [³H]GlcNAc but not [³H]man and was apparently resistant to the inhibitory effects of TM (Fig. 6)

Iodination of viral envelope proteins. Figure 7 illustrates the pattern of surface

envelope proteins of NOV and occluded AcNPV accessible to lactoperoxidase-catalyzed iodination. In control preparations without lactoperoxidase, there was a greater than 99% reduction in TCA-precipitable ¹²⁵I counts. We had previously determined (Stiles et al., 1983b) that labeling of nucleocapsid proteins is negligible under these conditions. A total of 13 NOV surface envelope proteins were labeled on virus produced by TN-368 cells while 12 proteins were identified when virus was produced in IPLB-21 cells (Fig. 7). A few additional bands were occasionally observed but not consistently enough to be included as viral proteins. These additional bands may represent contaminating cellular material. The asterisks beside certain of the proteins indicate envelope proteins which we had previously found to be glycosylated. The TN-368 cell derived nonoccluded virus contained a 100K molecular weight protein not seen in the IPLB-21 cell-derived virus. In addition, one viral protein (vp) band had a slightly different estimated molecular weight (vp 15 versus vp 14) from TN-368 and IPLB-21 cells, respectively. We also observed that the 38K viral glycoprotein which is present in NOV from both cell lines was surface labeled only in virus derived from IPLB-21 cells. Differences in protein glycosylation patterns may have blocked iodination of this glycoprotein in TN-368 cell-derived NOV. When iodination of the major surface proteins of TN-368 and IPLB-21 cells was performed, none of the host cell plasma membrane proteins observed (data not shown) matched the NOV proteins labeled in Fig. 7. However, difficulties in iodinating cell surface proteins make it impossible to completely rule out the presence of host membrane proteins in the nonoccluded virus.

Occluded virus purified from TN-368 cells had 14 envelope protein bands capable of being iodinated (Fig. 7) even though a substantial amount of occlusion body matrix protein (vp 33) remained with the virions after purification from the OBs.

DISCUSSION

The IPLB-21 cell line was chosen as the primary line for intracellular glycoprotein
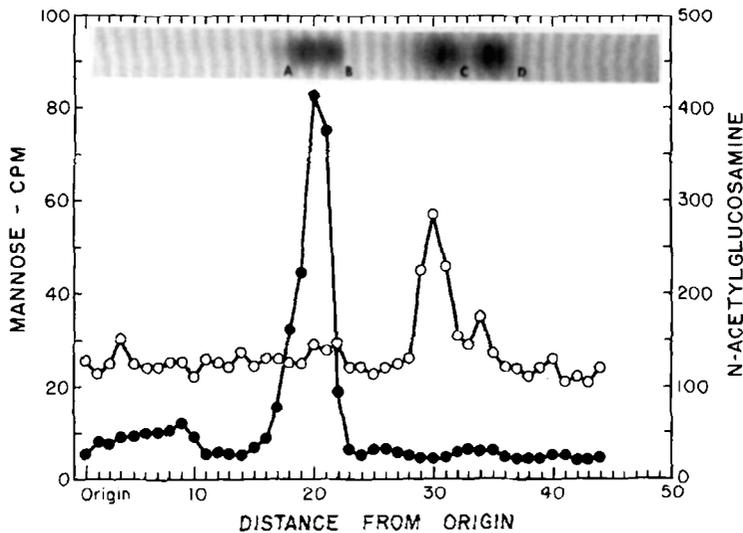
FIG. 5. Thin layer chromatographic separation of labeled sugars incorporated into AcNPV-NOV derived from IPLB-21 cells. Acid hydrolysis was used to liberate individual sugars and amino acids before chromatographic separation. (O) represents virus labeled with [³H]mannose while (●) represents virus labeled with N-[³H]acetylglucosamine. The strip across the top of the figure contains the unlabeled sugar standards visualized with silver stain (Krebs et al., 1969). A, glucosamine; B, galactosamine; C, mannose; D, fucose. (Note galactose and glucose run between B and C under these conditions.)

synthesis studies because in preliminary experiments (data not shown) control and infected IPLB-21 cells exhibited a much higher rate of incorporation of [³H]man in TCA-precipitable proteins than did TN-368 cells. Therefore, only IPLB-21 cells could be easily used for the short label times required in the time course experiments. When longer labeling periods were used during NOV production, both cell lines gave satisfactory levels of incorporation.

To ensure that the label added was being incorporated into viral proteins primarily as carbohydrate, the glycosylation inhibitor tunicamycin (TM) was added to infected cell samples at 0 hr pi. A concentration of 10 μg/ml was used because we had previously determined that at this level virus was still formed and released from cells although the virions were noninfectious (Stiles et al., 1983). When TM was present for more than 6 hr, it completely blocked the incorporation of label into virus protein bands (Fig. 1) indicating that metabolism of the labeled sugar was not significant under the stated experimental conditions. In addition, NOV pro-

duced in the presence of TM from either IPLB-21 or TN-368 cells did not label with either sugar. The fact that all of the viral-induced glycoproteins were sensitive to TM indicates that they are glycosylated via N-glycosidic bonds.

Nine intracellular viral-induced glycoproteins were identified when IPLB-21 cells were infected with AcNPV (Fig. 2). These glycoproteins appeared as early as 2 hr pi to as late as 14 hr pi. Once glycosylation of a viral protein began it continued throughout the course of the experiment (up to 28 hr pi).

There are five published studies of [³⁵S]methionine-labeled intracellular viral-induced proteins of either AcNPV or the closely related virus Trichoplusia ni NPV in either S. frugiperda or T. ni cells. Discrepancies in the number and time of appearance of viral proteins between the various studies are undoubtedly due to factors such as different multiplicities of infection used (Maruniak and Summers, 1981), masking of viral proteins by various host cell proteins, and varying amounts of radioactive label used. The intracellular
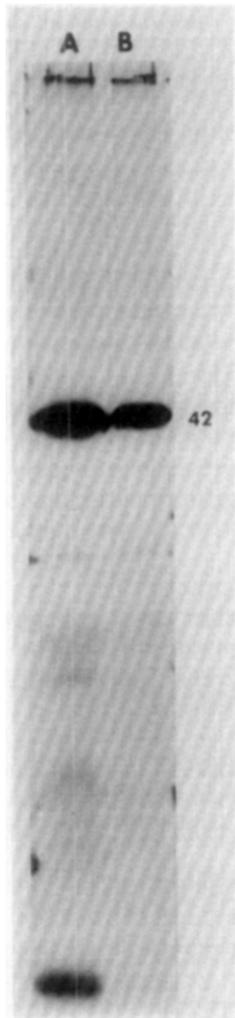
FIG. 6. AcNPV-occluded virus derived from TN-368 cells labeled with N-[³H]acetylglucosamine. A, normal virus; B, virus produced in the presence of 10 μg/ml TM (same approximate amount of virus as in lane A). The approximate molecular weight (×10⁻³) is indicated. The sample front in lane A also contained ³H label.

and Summers, 1981; Wood, 1980a). The other five viral proteins were observed to be glycosylated at approximately the same time as their synthesis was detected (identified with [³⁵S]methionine (Carstens *et al.*, 1979; Dobos and Cochran, 1980; Kelly and Lescott, 1981; Maruniak and Summers, 1981; Wood, 1980a)). Following the virus development sequence scheme proposed by Wood (1980a) for AcNPV, four virus glycoproteins (87K, 75K, 67K, and 62K) were identified during the early phase (2 to 8 hr pi). The other five glycoproteins were first observed to be glycosylated during the intermediate phase (10 to 14 hr pi).
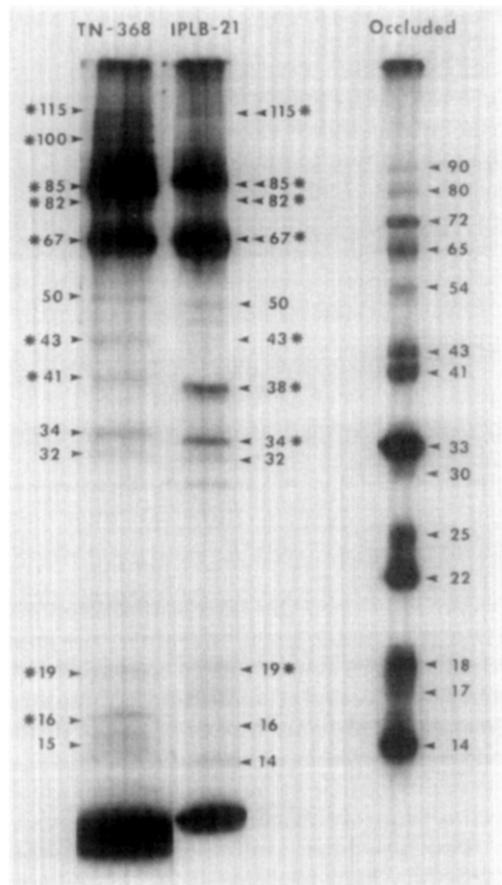
FIG. 7. Surface iodination of AcNPV NOV produced in IPLB-21 and TN-368 cells and AcNPV-occluded virus produced in TN-368 cells. Approximate molecular weights (×10⁻³) are indicated, arrows mark the proteins. The * indicates surface proteins that are glycosylated.

glycoproteins identified in this study corresponded to the [³⁵S]methionine-labeled intracellular viral proteins identified in three of the five previous studies. Four viral-induced glycoproteins, the 81K, 75K, 67K, and 62K, appeared slightly earlier when labeled with [³H]mannose than reported in [³⁵S]methionine-labeling studies (Carstens *et al.*, 1979; Dobos and Cochran, 1980; Kelly and Lescott, 1981; Maruniak

A comparison of AcNPV intracellular glycoproteins produced in IPLB-21 cells with those produced in TN-368 cells at 24 hr pi (Fig. 3) showed that the glycoprotein patterns were identical except that 87K and 34K glycoproteins were not observed in TN-368 cells. The 87K glycoprotein may have been masked by a heavily labeled host glycoprotein which had approximately the same mobility. The 34K glycoprotein was a very minor glycoprotein in IPLB-21 cells and we may simply have failed to detect it in the TN-368 cells.

When the intracellular viral glycoproteins are compared to the virus structural glycoproteins (Figs. 2–4 and Table 1) we see that 75K and 62K glycoproteins are strictly intracellular viral-induced glycoproteins (produced in both cell lines). In addition, there were a number of structural glycoproteins which were not identified in the intracellular experiments. This may have been due to masking by host cell glycoproteins of similar mobility, processing, or cleavage of some glycoproteins before their incorporation into virions, or they may represent a consistent contamination of NOV preparations by host cell glycoproteins.

Differences were observed between the glycoproteins of NOV derived from the two cell lines. Several animal virus glycoproteins have been shown to have altered mobility in polyacrylamide gels when the virus was grown in different host cells (Etchison et al., 1981; Keegstra and Burke, 1977; Simons and Garoff, 1980). The virus apparently uses the host's unaltered glycosylation machinery; therefore, the same viral protein can undergo very different types of glycosylation depending upon the host cell. This may account for the differences observed here although we may also be dealing with some contaminating host cell glycoproteins.

Two previous studies have identified structural glycoproteins of the nonoccluded form of AcNPV. Goldstein and McIntosh (1980) reported observing one glycoprotein of an approximate molecular weight of 70K in NOV derived from either IPLB-21 or *T. ni* TN-CL1 cells. This glycoprotein undoubtedly corresponds to the vpg 67 iden-

tified as the major structural glycoprotein in this study. Maruniak (1979) observed four high molecular weight vpg's which correspond to our vpg 115, vpg 85, vpg 82, and vpg 67. The lower molecular weight glycoproteins identified in this study were not reported by Maruniak. Possibly our procedure of adding label to infected cells at 10 hr pi after the virus has begun to suppress the host cell synthesis (Carstens et al., 1979) rather than at 0 hr pi (as did Maruniak (1979)) produces a more efficient labeling of the minor viral glycoproteins.

When attempts were made to label the occluded virus glycoproteins, a single 42,000 molecular weight band was observed only after labeling with N-acetylglucosamine (it did not label with mannose). This glycoprotein was also insensitive to TM treatment. We cannot at this time rule out the possibility that the 4-day labeling period used allowed the label to be metabolized and selectively incorporated into this structural protein. However, the fact that we did not see significant label transfer following shorter label periods and that the label showed up in a single occluded virus band suggests to us that this is a glycoprotein. In addition, its insensitivity to TM treatment and failure to label with [³H]man is presumptive evidence that this glycoprotein contains O-glycosidic bonds (mannose is generally not present in O-glycosidic glycoproteins—Niemann and Klenk (1981)). These data are consistent with the unaltered infectivity of AcNPV OBs produced in the presence of TM (Stiles et al., 1983a). O-Glycosidic glycoproteins have been detected in several other animal viruses (Holmes et al., 1981; Niemann and Klenk, 1981; Shida and Dales, 1981).

Maruniak (1979) and Dobos and Cochran (1980) reported 14 vpg's and 6 vpg's, respectively, in occluded AcNPV. In both studies the major vpg had a molecular weight of approximately 42K and was not labeled by [³H]man. The minor vpg's may have been detected because these authors added the tritiated sugars at 0 hr pi. However, this may also have allowed sufficient opportunity for the sugars to be metabolized before being incorporated into the virus proteins. Interpretation of the results

from Dobos and Cochran (1980) are complicated by the fact that they added a small proportion of insect-derived occlusion bodies containing an alkaline protease to aid in the release of the virus from the occlusion body matrix protein, which may account for the smaller number of glycoproteins than reported by Maruniak. It has since been demonstrated that the occlusion-body-associated alkaline protease causes pronounced changes in the pattern of baculovirus structural (Wood, 1980a) and envelope proteins (Stiles et al., 1983b).

We have reported in this study the first attempt to identify the surface envelope proteins of the nonoccluded form of a baculovirus using a lactoperoxidase iodination system (Hubbard and Cohn, 1972). We identified a total of 13 envelope proteins in the NOV derived from TN-368 cells (Fig. 7). Twelve of these proteins were also identified in NOV produced by IPLB-21 cells (Fig. 7). A majority of the NOV structural glycoproteins identified were also labeled by lactoperoxidase iodination (Table 1). These envelope glycoproteins are prime candidates for future studies of glycoprotein involvement in viral attachment and penetration. The other glycoproteins identified in this study may also be located within the viral envelope. Steric hindrance by the oligosaccharide side chains or a lack of tyrosine residues in the portions of the proteins exposed on the outer surface of the envelope may explain their lack of iodination. Of the noniodinated glycoproteins only vpg 37 or 38 may correspond to one of the AcNPV nucleocapsid proteins which were identified by Summers and Smith (1978).

Iodination of AcNPV released from TN-368 cell-derived occlusion bodies resulted in the labeling of 14 proteins—envelope proteins which have a portion of their molecules exposed on the outer surface of the viral envelope. We cannot at this time be certain whether the 42K molecular weight glycoprotein identified corresponds to an envelope protein (either the 41K or 43K protein) or represents a capsid protein.

In conclusion, we have identified the intracellular and virus structural glycopro-

TABLE 1

COMPARISON OF INTRACELLULAR AND NOV STRUCTURAL GLYCOPROTEINS (GPs) OF
AcNPV (1A CLONE) FROM IPLB-21 AND TN-368 CELLS

Time (hr)	IPLB-21 Cells		TN-368 Cells	
	Intracellular viral GPs (Mol wt $\times 10^{-3}$)	Structural viral GPs (Mol wt $\times 10^{-3}$)	Intracellular viral GPs (Mol wt $\times 10^{-3}$)	Structural viral GPs (Mol wt $\times 10^{-3}$)
—	—	125	—	125
—	—	115[a]	—	115[a]
—	—	—	—	100
8	87	85[a]	—	85[a]
—	—	82[a]	—	82[a]
6	75	—	75	—
2	67	67[a]	67	67[a]
8	62	—	62	—
—	—	43[a]	—	43[a]
—	—	—	—	41[a]
—	—	38[a]	—	38
10	37	37	37	—
14	36	36	36	—
12	34	34[a]	—	—
14	19	19[a]	19	19
—	—	—	16	16[a]

[a] Envelope glycoproteins labeled by lactoperoxidase-catalysed surface radioiodination.

teins of AcNPV. We found that TM treatment inhibited the glycosylation of all of the NOV glycoproteins. This lack of glycosylation is presumably responsible for the loss of infectivity of budded virus previously observed after treatment with TM (Stiles *et al.*, 1983a). The production of noninfectious virus particles in the presence of TM has been reported with several other virus systems (Roth *et al.*, 1979; Stohrer and Hunter, 1979; Svennerholm *et al.*, 1982). The demonstration that the majority of NOV glycoproteins are located in the viral envelope with portions of their molecules exposed to the environment suggests that the glycoproteins may be involved in the attachment and penetration of the nonoccluded form of baculoviruses in cell culture. The observed TM insensitivity of the single glycoprotein of the occluded virus may explain the observation that tissue-culture-derived occlusion bodies produced in the presence of TM are fully as infectious to *T. ni* larvae as are control occlusion bodies (Stiles *et*

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