JOURNAL OF VIROLOGY, Aug. 1980, p. 362-370
0022-538X/80/08-0362/09$02.00/0

Vol. 35, No. 2

Altered Synthesis and Processing of Oligosaccharides of Vesicular Stomatitis Virus Glycoprotein in Different Lectin-Resistant Chinese Hamster Ovary Cell Lines

LAWRENCE A. HUNT†

Department of Microbiology, University of Kansas Medical Center, Kansas City, Kansas 66103

To determine the particular intracellular steps in the glycosylation of the vesicular stomatitis virus (VSV) glycoprotein that were altered in several lectin-resistant CHO cell lines, VSV-infected parental and mutant cells were pulse-labeled for 30 and 120 min with [^{3}H]mannose and [^{3}H]glucosamine. Cell-associated viral glycopeptides were analyzed by gel filtration combined with specific glycosidase digestions and compared with the corresponding mature virion oligosaccharides. The intracellular glycosylation of the VSV glycoprotein in a mutant cell line resistant to phytohemagglutinin was identical to that in the normal cells except for a complete block in processing at a specific step in the final trimming of the oligomannosyl core from five to three mannoses. The results demonstrated that a double-mutant cell line selected from the phytohemagglutinin-resistant cells for resistance to concanavalin A had an additional defect in one of the earliest stages of glycosylation, resulting in smaller precursor oligosaccharides linked to protein.

The vesicular stomatitis virus (VSV)-infected animal cell is an excellent experimental system for elucidating the molecular and subcellular mechanisms involved in the biosynthesis and maturation of membrane glycoproteins because VSV: (i) has an extremely wide host range; (ii) shuts off host protein synthesis; and (iii) contains a single, well-characterized species of glycoprotein (33). The VSV G polypeptide contains two major glycosylation sites with complex "acidic" oligosaccharides that are linked N-glycosidically to asparagine (Fig. 1, top) (3, 15, 18, 20). The biosynthesis and maturation of these oligosaccharide moieties involve two major steps (8–10, 19, 28): (i) en bloc transfer of large oligomannosyl cores from lipid-linked intermediates to the G polypeptide in the rough endoplasmic reticulum; and (ii) subsequent trimming of the oligomannosyl core from nine to three mannoses and addition of branch sugars (NeuNAc-Gal-GlcNAc-) in the smooth endoplasmic reticulum/Golgi membranes.

VSV has been used extensively as a specific probe for altered protein glycosylation in human cell lines from individuals with genetic diseases with unknown biochemical defects (11, 23) and in mutant cell lines selected for resistance to one or more plant lectins (21, 22, 28). Previous comparative studies with one particular group of lectin-resistant CHO cell lines demonstrated major alterations in the oligosaccharide moieties of the mature virion-associated G protein that presumably reflected the biochemical deficiencies of the particular mutant host cells (21; J. R. Etchison and D. F. Summers, J. Supramol. Struct. Suppl. **3**:205, 1979). The asparaginyl-oligosaccharide structures elucidated for VSV grown in the parental cell line (CHO-Parent), a single mutant resistant to phytohemagglutinin (Pha) (CHO-Pha^r) and a double mutant resistant to both Pha and concanavalin A (ConA) (CHO-Pha^r ConA^r) are shown in Fig. 1.

The absence of branch sugars and the increased number of mannose units (five versus three) in the oligomannosyl core of the viral oligosaccharides synthesized in the CHO-Pha^r host cells were consistent with the deficiency in a particular *N*-acetylglucosamine transferase in these cells (16, 25) and the necessity of adding the first branch *N*-acetylglucosamine before the removal of the final two mannose units (27). However, the biochemical defect(s) responsible for the additional alteration in the oligomannosyl core of the viral oligosaccharides synthesized in the CHO-Pha^r ConA^r host cells could not be predicted from the analysis of mature oligosaccharides from purified virus.

In this study, VSV-infected CHO-Parent, CHO-Pha^r, and CHO-Pha^r ConA^r cells were radiolabeled for various time periods with [^{3}H]-mannose, and the cell-associated viral glycopeptides and oligosaccharides were analyzed and compared with mature virion glycopeptides and

† Present address: Department of Microbiology and Immunology, University of Louisville Health Sciences Center, Louisville, KY 40232.

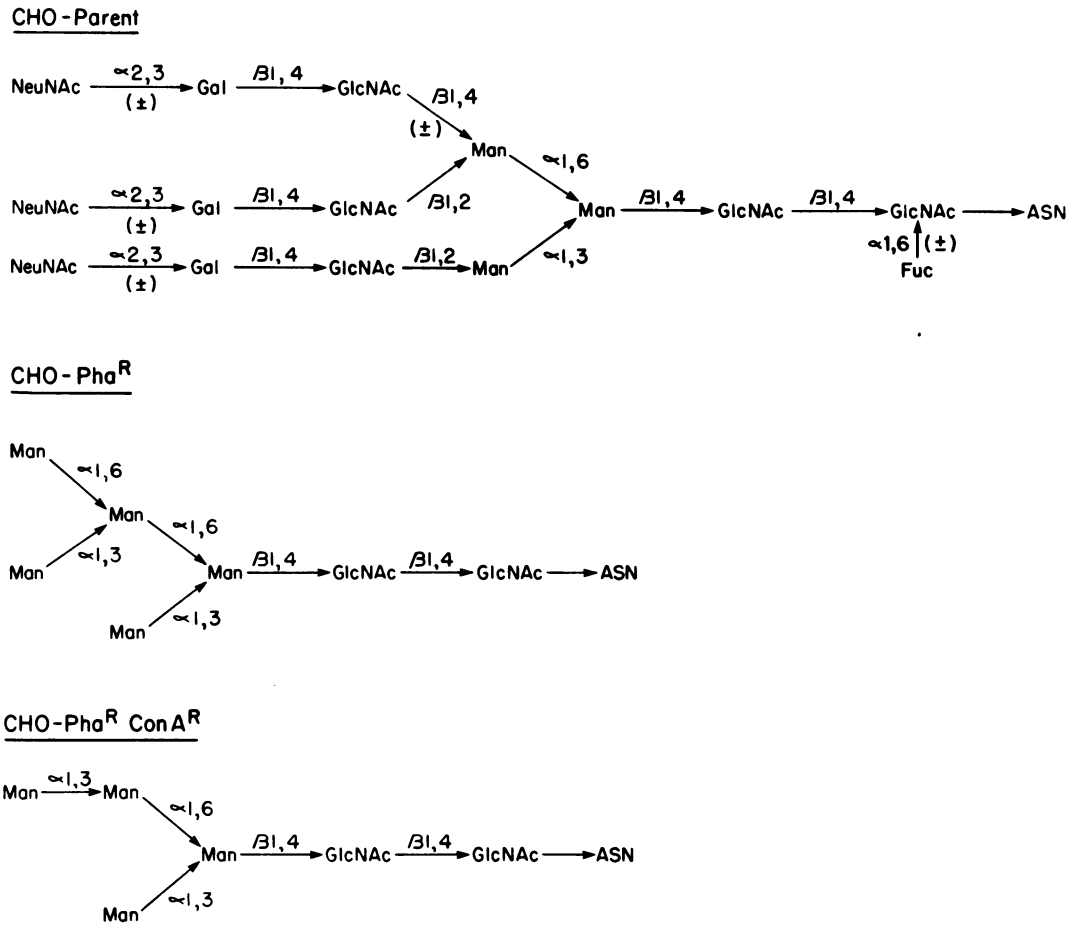

FIG. 1. *Structures of the carbohydrate moieties of the glycoprotein from VSV grown in the parental and lectin-resistant CHO cell lines. The structure shown for the parental cell-derived viral oligosaccharides is identical to that reported for the G protein of the New Jersey strain of VSV (18) and is consistent with the structure reported for G from VSV (Indiana) (3, 21). The structures for the lectin-resistant cell-derived viral oligosaccharides were previously described (21; Etchison and Summers, J. Supramol. Struct. Suppl. 3:205, 1979).*

oligosaccharides from virus grown in the corresponding host cells. The results of these experiments demonstrated that the additional defect in the double mutant (CHO-Pha^r ConA^r) involved smaller than normal precursor oligosaccharides for the VSV G protein. In addition, the results confirmed the previously observed alterations in mature viral oligosaccharides and indicated that the glycosylation of the G protein in the single mutant (CHO-Pha^r) was identical to that in the normal cells except for the complete block of processing at a specific step in the final trimming of the oligomannosyl core.

MATERIALS AND METHODS

Cells, virus, and radioactive labeling. The CHO parental and mutant cell lines were obtained from Pamela Stanley, Albert Einstein College of Medicine. The cells were grown in monolayer culture at 37°C in Eagle minimal essential medium (KC Biological) containing 10% fetal bovine serum (KC Biological) supplemented with 40 mg of proline and 10 mg of each of glycine, adenosine, and thymidine per liter. The parental cell line was Gat$^-$2 (auxotroph requiring glycine, adenosine, and thymidine). The mutant cell lines were Gat$^-$ Pha^{rI} (clone selected from Gat$^-$2 for resistance to Pha) and Gat$^-$ Pha^{rI} ConA^{rII}3B (clone selected from Gat$^-$Pha^{rI} for resistance to ConA). Their nomenclature and genetic characterization have been previously described in more detail (24, 26). The names of the cell lines have been shortened to CHO-Parent, CHO-Pha^r, and CHO-Pha^r ConA^r (21).

VSV (Indiana) was grown in HeLa S3 suspension cultures or BHK-21 monolayer cultures and purified as described previously (3, 9, 20). CHO cell cultures were infected with approximately 10 PFU of purified VSV per cell and radiolabeled at 4 h after infection with 100 μCi of [2-^{3}H]mannose per ml (Amersham

Corp., Arlington Heights, Ill.; 2 Ci/mmol) in glucose-deficient medium as previously described (8, 10). Radiolabeled VSV was also purified from VSV-infected CHO cells labeled overnight (3 to 20 h postinfection) with 20 μCi of [2-^{3}H]mannose per ml (10, 20).

Preparation and analysis of membrane glycoproteins. Radiolabeled glycoprotein was extracted with *n*-butanol from Nonidet P-40 detergent-treated virus and cell homogenates and digested with pronase as described earlier (8, 10). Glycopeptides were analyzed by gel filtration through Bio-Gel P-4 (−400 mesh) columns along with various gel filtration markers (8, 10). Glycopeptides were desalted on a Sephadex G-15/G-50 column (10) before glycosidase digestions.

Glycopeptides and oligosaccharides were digested with exoglycosidases and endo-β-*N*-acetylglucosaminidases as previously described (3, 8). Endo-β-*N*-acetylglucosaminidase H (endo-H) from *Streptomyces plicatus* and the mixture of exoglycosidases and endo-β-*N*-acetylglucosaminidase D (endo-D) from *Streptococcus pneumoniae* were provided by James Etchison, University of Utah. Endo-H was also purchased from Miles Laboratories (Elkhart, Ind.). The oligomannosyl neutral cores and residual glycopeptide products of endo-H and endo-D digestions were separated by passing the digests through a column of Dowex AG-X2 (formate) as described elsewhere (21). The elution positions of various neutral oligomannosyl cores ($Man_nGlcNAc_1$) of known composition were used to calibrate the Bio-Gel P-4 columns in the gel filtration of endo-H and endo-D digestion products (3, 8, 21).

RESULTS

Viral glycoprotein from VSV-infected CHO cells and released virus. The VSV G protein was the only major radiolabeled species observed after labeling of cells and virus with [^{3}H]mannose (Fig. 2). Both the cell-associated and released virion glycoprotein from the two lectin-resistant cell lines (CHO-Pha^r, CHO-Pha^r ConA^r) exhibited an increase in electrophoretic mobility in sodium dodecyl sulfate-polyacrylamide gels compared with the glycoprotein from the parental cells, as previously reported for [^{35}S]methionine and [^{3}H]glucosamine-labeled G protein from the same lectin-resistant cell lines (21) or from a ricin-resistant CHO cell line with a phenotype identical to that of CHO-Pha^r (22). A minor radiolabeled glycoprotein with slightly greater electrophoretic mobility than VSV G protein was observed in some of the cell-associated samples. A similar species has been previously detected in VSV-infected HeLa cells (8, 10).

These observations were consistent with the alterations in carbohydrate structures for the mature viral glycoprotein shown in Fig. 1. Differences in the electrophoretic mobility of glycoprotein from the corresponding VSV-infected cells after a 30-min pulse-label with [^{3}H]mannose were less significant (data not shown), suggesting that the precursor oligosaccharides of newly synthesized viral glycoprotein were similar in the three CHO cell lines.

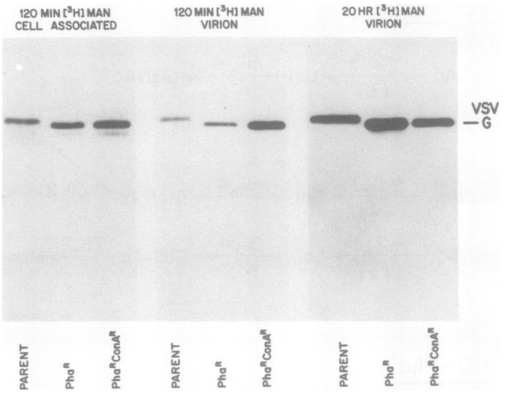

FIG. 2. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [^{3}H]mannose-labeled polypeptides from VSV-infected parental and lectin-resistant CHO cells and released virus. Cell homogenates or viruses were subjected to electrophoresis on a 10% polyacrylamide slab gel as previously described (9). Bands corresponding to radiolabeled glycoproteins were detected by fluorography of the dried gel (1). The labeling time and radioactive precursor are indicated above each gel profile, and the source of the virus or cell homogenate is indicated below.*

Analysis of viral glycopeptides from VSV-infected CHO-Parent and CHO-Pha^r cells. To compare the intracellular biosynthesis and maturation of the VSV glycoprotein oligosaccharides in the Pha-resistant cells and the parental cells from which these mutant cells had been derived, [^{3}H]mannose-labeled glycopeptides were prepared from homogenates of VSV-infected cells after different pulse-labeling periods and from purified virus labeled overnight. Figure 3 shows the Bio-Gel P-4 elution profiles of glycopeptides from the 30- and 120-min labeling of the VSV-infected CHO-Parent cells and the corresponding released virus. Most of the radiolabeled glycopeptides from the 30-min (Fig. 3A) and 120-min (Fig. 3B) labeled cells eluted in two partially resolved peaks in the vicinity of the ^{14}C-labeled glycopeptide marker (fractions 60 through 75). Apparently higher-molecular-weight, mature-size glycopeptides (S_1 through S_3) increased in relative amount in the 120-min sample compared with the 30-min sample. The pronase digest of purified virus contained only these mature-size glycopeptides (Fig. 3C).

When equal portions of these same glycopeptide samples were digested with endo-H (an enzyme capable of cleaving between the two *N*-acetylglucosamine residues proximal to the asparagine in mannose-rich, neutral oligosaccha-

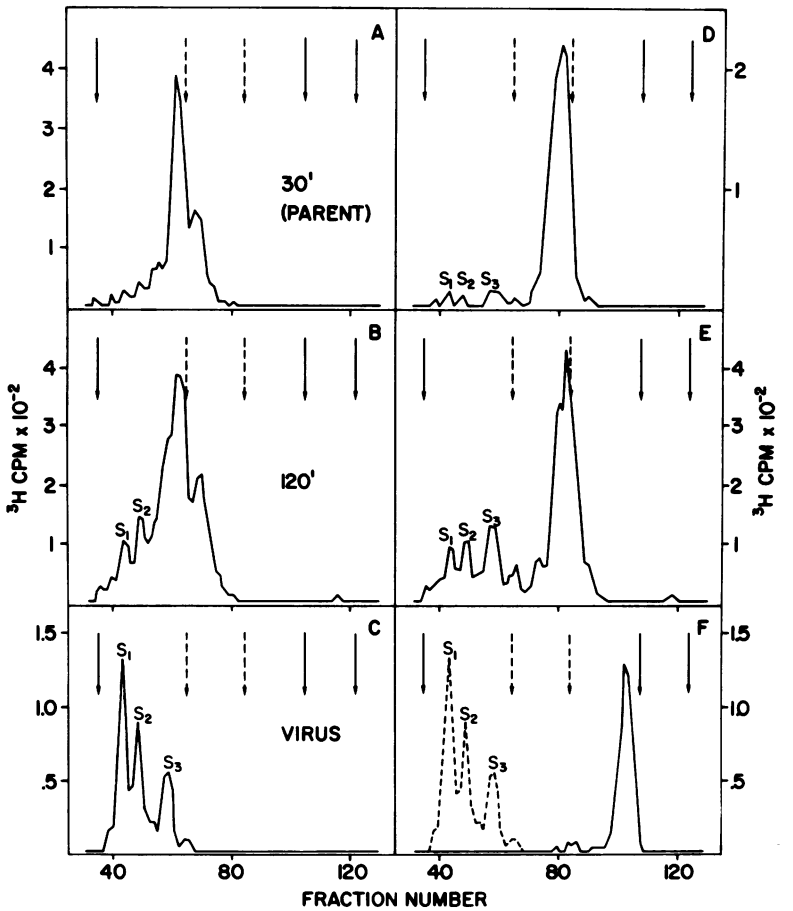

FIG. 3. *Bio-Gel P-4 filtration of untreated and glycosidase-treated glycopeptides from [^{3}H]mannose-labeled VSV-infected CHO-Parent cells and released VSV. Glycopeptides from 30-min and 120-min radiolabeled cells were analyzed either before (A,B) or after (D,E) treatment with endo-H. Glycopeptides from virus radiolabeled overnight were analyzed either before (C) or after (F) treatment with endo-D and the exoglycosidase mixture. The glycopeptides from released virus were insensitive to endo-H digestion (profile shown by dotted line in F). The solid arrows indicate, from left to right, the peak elution positions of blue dextran 2000 (void volume; molecular weight, approximately 4,000 or greater), stachyose (molecular weight, 666), and mannose (molecular weight, 180). The broken arrows indicate the peak elution position of Man$_5$GlcNAc$_2$ASN (acetyl-^{14}C) and GlcNAc-ASN (acetyl-^{14}C) cochromatographed with the ^{3}H-labeled glycopeptides. These ^{14}C-labeled structures were not reliable molecular weight markers because of the negative charge exclusion property of Bio-Gel resins (3, 10). S_1, S_2, and S_3 correspond to mature VSV glycopeptides (Fig. 1, top) that differ in the amount of terminal sialic acid (2, 1, and 0 residues, respectively [3, 10]).*

ride structures [29, 31]), radiolabel in the major peaks of cell-associated glycopeptides was shifted to lower-molecular-weight fractions (Fig. 3D, E).

As expected, the radiolabel in mature-size glycopeptides was unchanged in elution position (Fig. 3D,E) and appeared in S_1-, S_2-, and S_3-size peaks similar to the endo-H-resistant glycopeptides from purified virus (Fig. 3F). These glycopeptides were shown to contain a small oligomannosyl core (Man$_3$GlcNAc$_1$-size) after digestion with a mixture of exoglycosidases and endo-D, an enzyme with a specificity different from that of endo-H (30) (Fig. 3F). These results were essentially identical to those previously reported for [^{3}H]mannose-labeled viral glycopeptides from VSV-infected HeLa and BHK cells (8, 10): precursor oligosaccharides were the predominant radiolabeled structures linked to glycoprotein after pulse-labeling for 15 to 30 min, and a mixture of precursor and mature oligosaccharide structures was obtained after 60- to 120-min labeling periods. In addition, the absence of significant radiolabel in structures other than the

neutral oligomannosyl cores after exoglycosidase and endo-D and endo-H digestions indicated that essentially all of the ^3H label incorporated into viral protein was in the form of mannose.

The results of an analogous experiment with viral glycopeptides from VSV-infected CHO-Pha^r cells and purified virus released from these cells are shown in Fig. 4, with the profiles of endo-H-digested glycopeptides super-imposed for the corresponding cell-associated and virion samples from the CHO-Parent and CHO-Pha^r host cells. Essentially all of the radiolabel in viral glycopeptides from the CHO-Pha^r cells was sensitive to endo-H, with the removal of the peptide moiety confirmed by Dowex ion-exchange chromatography (data not shown).

The precursor oligosaccharides were apparently identical for the viral glycopeptides from the CHO-Parent and CHO-Pha^r cells (Fig. 4A). After 120 min of labeling, mature S_1–S_3-size glycopeptides accumulated in the CHO-Parent cells, but only the intermediate-size oligomannosyl cores accumulated in the CHO-Pha^r cells. These $Man_5GlcNAc_1$-size oligosaccharides were the only major species present in the endo-H digest of glycopeptides from purified virus released from the CHO-Pha^r cells (Fig. 4C). No significant amount of radiolabel accumulated between the precursor- and mature-size oligomannosyl core structures in the virus-infected CHO-Pha^r cells or in smaller oligomannosyl cores in the virus-infected CHO-Parent cells.

Comparison of viral glycopeptides from VSV-infected CHO-Pha^r ConA^r and CHO-Pha^r cells. The glycopeptides from 30- and 120-min [^3H]mannose-labeled CHO-Pha^r ConA^r cells and purified virus were analyzed in a manner identical to the analysis of the corresponding glycopeptides from the other VSV-infected CHO cells. Obvious differences in the gel filtration profiles for the VSV-infected CHO-Pha^r and CHO-Pha^r ConA^r samples were observed when the profiles of endo-H-digested glycopeptides from the corresponding cell-associated and virion samples were superimposed by alignment of the gel filtration column markers (Fig. 5). A comparison with the profiles of the untreated glycopeptides (data not shown) indicated that the CHO-Pha^r ConA^r cells were also devoid of larger, endo-H-resistant glycopeptides. The elution positions of the major species of virion oligosaccharides (Fig. 5C) were the same as neutral oligomannosyl cores ($Man_nGlcNAc_1$) with five and four mannose units, respectively, for the CHO-Pha^r and CHO-Pha^r ConA^r host cells, as previously reported for [^3H]- and [^{14}C]glucosamine-labeled glycopeptides from VSV grown in these same lectin-resistant cell lines (21).

The most important difference was the smaller size of the predominant radiolabeled peak from the endo-H-digested, 30-min radiolabeled glycopeptides of the CHO-Pha^r ConA^r cells (Fig. 5A). This result indicated that the precursor oligomannosyl core structures were smaller than normal by several hexose units.

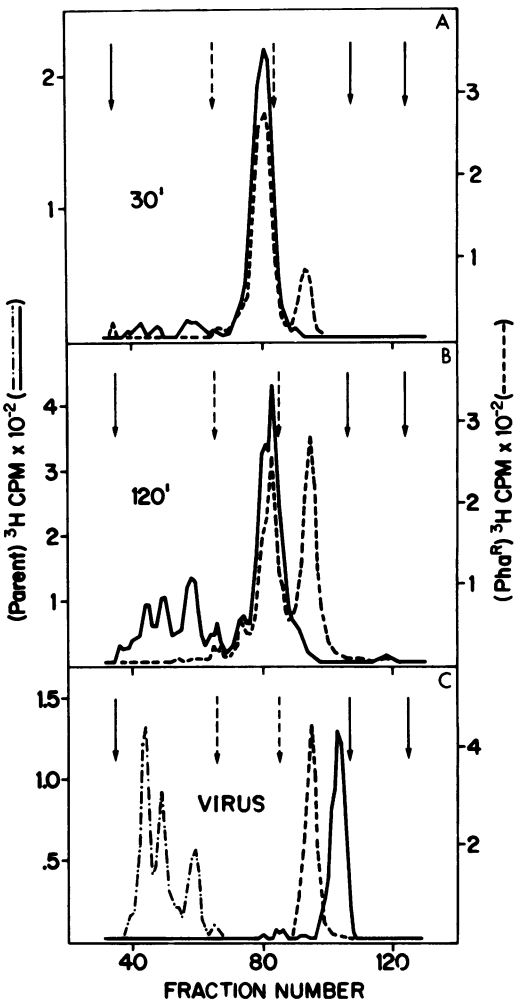

FIG. 4. *Comparison of Bio-Gel P-4 gel filtration profiles of endo-H-treated, [^3H]mannose-labeled glycopeptides from VSV-infected CHO-Parent and CHO-Pha^r cells and released virus. Profiles of VSV-infected CHO-Parent glycopeptides* (——) *and VSV-infected CHO-Pha^r glycopeptides* (·····) *were superimposed by alignment of the peak elution positions of the five column markers. (A) 30-min radiolabeled cell-associated glycopeptides; (B) 120-min radiolabeled cell-associated glycopeptides; (C) glycopeptides from released virus. The profiles of both the endo-H-resistant glycopeptides* (-·-·-·-) *and endo-D and exoglycosidase-digested glycopeptides* (——) *from VSV grown in the CHO-Parent cells are shown in (C).*

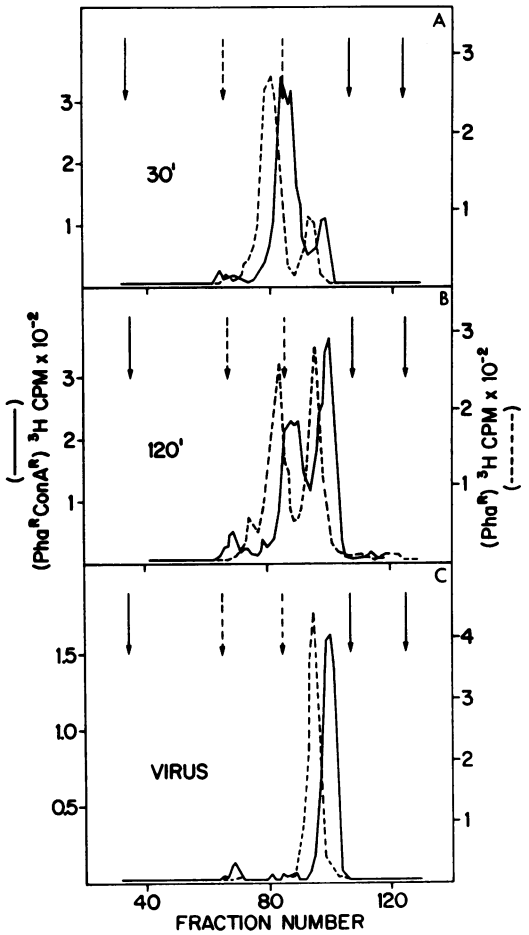

FIG. 5. *Comparison of Bio-Gel P-4 gel filtration profiles of endo-H-treated [^{3}H]mannose-labeled glycopeptides from VSV-infected CHO-Pha^r and CHO-Pha^r ConA^r cells and released virus. Profiles from VSV-infected CHO-Pha^r glycopeptides (-----) and VSV-infected CHO-Pha^r ConA^r glycopeptides (——) were superimposed by alignment of the peak elution positions of the five column markers. (A) 30-min radiolabeled cell-associated glycopeptides; (B) 120-min radiolabeled cell-associated glycopeptides; (C) glycopeptides from released virus.*

With the longer labeling period, the relative amount of radiolabel in these precursor oligosaccharides decreased and the relative amount in mature virion-size oligosaccharides increased for both the CHO-Pha^r and CHO-Pha^r ConA^r samples. These studies and additional studies with [^{3}H]glucosamine-labeled VSV-infected CHO cell glycopeptides (data not shown) suggested that the additional defect(s) in the VSV-infected CHO-Pha^r ConA^r cells that resulted in a smaller mature oligosaccharide for the VSV G protein ($Man_4GlcNAc_2$-ASN versus $Man_5GlcNAc_2$-ASN in CHO-Pha^r cells) was in the initial rather than in later stages of glycosylation when mannose trimming was completed.

The presence or absence of terminal glucose may have been responsible for heterogeneity in the precursor oligosaccharides linked to viral protein in the different VSV-infected CHO cells, since removal of glucose occurs soon after transfer of the complete precursor oligosaccharide ($Glc_3Man_9GlcNAc_2$) from lipid to protein (7, 14, 19, 28). To better examine this possible heterogeneity, the analyses of the endo-H-digested samples from the 30-min VSV-infected CHO cells were repeated on a newer Bio-Gel P-4 column that was capable of improved resolution of a series of neutral oligosaccharide species ($Man_nGlcNAc_1$, with n = 3 to 9). The precursor oligosaccharides from both the CHO-Parent and CHO-Pha^r cells eluted from the column as a doublet that was consistent in size with the $Glc_1Man_9GlcNAc_1$ and $Man_9GlcNAc_1$ oligosaccharides demonstrated elsewhere (7) to be the major products of endo-H-digested glycopeptides from chicken embryo fibroblasts after similar [^{3}H]mannose labeling for 30 min (Fig. 6A, B).

A corresponding doublet was also observed in the gel filtration profile of the viral oligosaccharides from the CHO-Pha^r ConA^r host cells (Fig. 6C), with both the larger and smaller of the two peaks eluting after the two size classes of precursor oligosaccharides from the CHO-Parent and CHO-Pha^r cells. The gel filtration properties of these smaller precursor oligosaccharides were consistent with $Man_7GlcNAc_1$ for the smaller species and either $Glc_1Man_7GlcNAc_1$ or $Man_8GlcNAc_1$ for the larger species.

DISCUSSION

The gel filtration analysis of [^{3}H]mannose glycopeptides from VSV-infected CHO parental, CHO-Pha^r, and CHO-Pha^r ConA^r cell lines has permitted a clearer understanding of the defects in intracellular glycosylation processes that result in the previously described alterations (21) in the asparagine-linked oligosaccharide moieties of the VSV glycoprotein. The comparisons of cell-associated viral glycopeptides and released virion glycopeptides confirmed the previously determined mature viral oligosaccharide structures shown in Fig. 1. Furthermore, the results demonstrated the absence of viral oligosaccharides in the lectin-resistant cells that were more fully processed than the mature oligosaccharides associated with virus released from these cells. The absence of branch sugars characteristic of complex acidic oligosaccharides (NeuNAc-Gal-GlcNAc-) and the presence of the larger oligomannosyl core (five versus three mannoses) in the mature oligosaccharides asso-

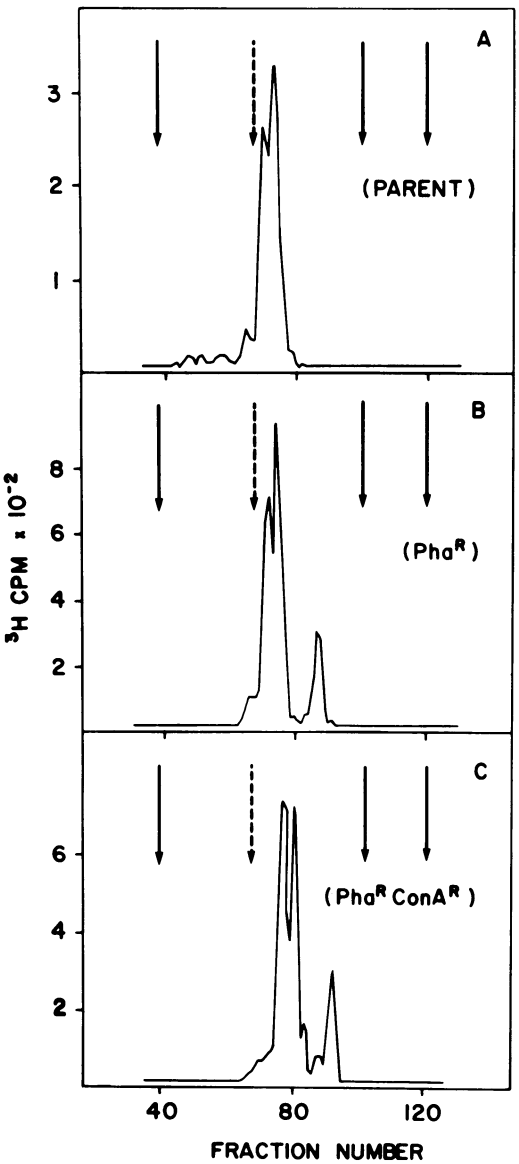

FIG. 6. *Bio-Gel P-4 gel filtration of endo-H-treated glycopeptides from 30-min [^{3}H]mannose-labeled, VSV-infected parental and lectin-resistant CHO cells. The three samples (A–C) correspond to the samples displayed previously in Fig. 4A and 5A. The gel filtration standards (minus lower-molecular-weight ^{14}C-labeled marker) were identical to those in Fig. 3, but the column was poured from a newer batch of Bio-Gel P-4 and displayed increased resolution of endoglycosidase-released neutral oligosaccharides ($Man_nGlcNAc_1$).*

ciated with both the infected CHO-Pha^r cells and released virus were consistent with the deficiency in the specific *N*-acetylglucosamine transferase (16, 25) being the only biochemical defect in these cells. An apparently identical biochemical defect has been demonstrated in a ricin-resistant CHO cell line designated "clone 15B" (5). Studies with clone 15B and normal cells indicated that the removal of the final two mannose units from the VSV asparaginyl oligosaccharides was coupled to the addition of the first branch *N*-acetylglucosamine by this specific transferase (27).

The most important result of the experiments presented here is the assignment of the additional defect in the VSV-infected CHO-Pha^r ConA^r cells to a smaller precursor oligomannosyl structure for the viral glycoprotein, as opposed to an additional α-mannosidase activity in the final stage of processing suggested elsewhere (27). From the results presented here, one cannot conclude whether the defect is in the actual synthesis of a smaller precursor oligosaccharide or in aberrant early processing of a normal precursor oligosaccharide. More recent and detailed studies with both the lipid-linked and peptide-linked precursor oligosaccharides of the corresponding uninfected CHO cell lines were undertaken to resolve this question and have demonstrated that the defect in the CHO-Pha^r ConA^r cells is the synthesis and transfer to protein of smaller (seven versus nine mannoses) precursor oligosaccharides (L. A. Hunt, manuscript submitted for publication). These newer studies also indicated that the major species of protein-linked precursor oligosaccharides from 30-min [^{3}H]mannose-labeled CHO cells were identical in endoglycosidase sensitivity and gel filtration properties to the viral glycopeptides in these studies and had the following composition: $Glc_{0-1}Man_7GlcNAc_2$-ASN for the CHO-Pha^r ConA^r cells and $Glc_{0-1}Man_9GlcNAc_2$-ASN for the CHO-Parent and CHO-Pha^r cells.

Although the exact biochemical defect responsible for the smaller precursor oligosaccharides is not known at present, one logical explanation is the absence of a specific α-1,6-mannosyltransferase. This idea is based upon the difference between the mature viral oligosaccharides for VSV grown in the CHO-Pha^r versus CHO-Pha^r ConA^r cells (Fig. 1) and the presumed absence of the specific α-1,6-linked mannose from the precursor as well as mature oligosaccharides from the VSV-infected CHO-Pha^r ConA^r cells. The biochemical defect(s) in another CHO mutant cell line selected in the first step for resistance to ConA seems to be distinct (12), because the CHO-Pha^r ConA^r mutant can complement this CHO-ConA^r mutant in cell hybridization studies (26). The altered precursor oligomannosyl structures in the VSV-infected CHO-Pha^r ConA^r cells (endo-H sensitive) are obviously different from these found in a mutant murine

lymphoma cell line with a ConA-resistant phenotype (five mannoses and endo-H resistant) (2, 32).

The processing of the oligosaccharides of the viral glycoprotein in the [^{3}H]mannose-labeled VSV-infected CHO-Parent cells was essentially identical to that observed earlier for VSV-infected HeLa and BHK cells (8): the trimming of mannoses from the precursor oligomannosyl core and the addition of *N*-acetylglucosamine, galactose, and sialic acid occurred almost simultaneously, so that no significant amount of radiolabel was observed in partially trimmed, intermediate structures (Fig. 3). This was different from experimental results reported elsewhere for VSV-infected chicken embryo fibroblast cells, in which significant amounts of an intermediate structure ($Man_5GlcNAc_2$-peptide) were observed in addition to the precursor glycopeptides with large oligomannosyl cores and mature S_1-S_3-size glycopeptides (7). Once the trimming of the precursor oligomannosyl core structures for the VSV G protein was initiated in the CHO cells, the mannose removal proceeded rapidly to completion until blocked in the two lectin-resistant cell lines by the *N*-acetylglucosaminyltransferase defect, since little or no [^{3}H]mannose label was observed in oligosaccharides intermediate in size between oligomannosyl precursor and four- to five-mannose mature structures (Fig. 5 and 6). The small amount of intermediate-size structures in the 30- and 120-min labeled double-mutant cells might be explained by a possible alteration in the rate and completion of mannose removal caused by the altered precursor core structure, or by a background of radiolabeled cellular glycoprotein that possessed mature neutral oligosaccharides with five to six mannoses.

One major question left unanswered by these investigations is the function of the multiple and elaborate steps of oligosaccharide biosynthesis and processing for membrane glycoproteins such as the VSV G protein. Major alterations in the processing and final composition of the viral oligosaccharides can occur without significant differences in the efficiency of viral assembly or the infectivity of the resulting viral particles (21, 22). In studies where the addition of oligosaccharides was completely eliminated by the addition of tunicamycin, the nonglycosylated G protein was relatively insoluble and was incorporated into mature infectious virions with a greatly reduced efficiency, but the resulting virions were just as infectious as those containing fully glycosylated G protein (4, 13). Some significance for specific oligosaccharide structures and processing in the interactions of cells with enveloped animal viruses has been suggested, however, in two more recently published studies with lectin-resistant cell lines: the clone 15B ricin-resistant CHO cell line (equivalent in phenotype to the CHO-Pha^r cells) was relatively insensitive to fusion induced by Newcastle disease virus (17); and two different ricin-resistant mouse L cell lines were restricted in their ability to replicate Sindbis and Semliki Forest viruses (6).

ACKNOWLEDGMENTS

I thank Marilyn Levy Murrmann for her excellent technical assistance, Pamela Stanley (Albert Einstein College of Medicine) for providing the parental and lectin-resistant CHO cell lines, and Brenda Smith for typing of the manuscript.

This work was supported by Public Health Service grant AI-14757 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method of tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
2. **Chapman, A., I. S. Trowbridge, R. Hyman, and S. Kornfeld.** 1979. Structure of the lipid-linked oligosaccharides that accumulate in class E thy-1 negative mutant lymphomas. Cell **17**:509–515.
3. **Etchison, J. R., J. S. Robertson, and D. F. Summers.** 1977. Partial structural analysis of the oligosaccharide moieties of the vesicular stomatitis virus glycoprotein by sequential chemical and enzymatic degradation. Virology **78**:375–393.
4. **Gibson, R., R. Leavitt, S. Kornfeld, and S. Schlesinger.** 1978. Synthesis and infectivity of vesicular stomatitis virus containing nonglycosylated G protein. Cell **13**:671–679.
5. **Gottlieb, C., J. Baenziger, and S. Kornfeld.** 1975. Deficient uridine diphosphate-N-acetylglucosamine: glycoprotein N-acetylglucosaminyl-transferase activity in a clone of Chinese hamster ovary cells with altered surface glycoproteins. J. Biol. Chem. **250**:3303–3309.
6. **Gottlieb, C., S. Kornfeld, and S. Schlesinger.** 1979. Restricted replication of two alphaviruses in ricin-resistant mouse L cells with altered glycosyltransferase activities. J. Virol. **29**:344–351.
7. **Hubbard, S. C., and P. W. Robbins.** 1979. Synthesis and processing of protein-linked oligosaccharides in vivo. J. Biol. Chem. **254**:4568–4576.
8. **Hunt, L. A., J. R. Etchison, and D. F. Summers.** 1978. Oligosaccharide chains are trimmed during synthesis of the envelope glycoprotein of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **75**:754–758.
9. **Hunt, L. A., and D. F. Summers.** 1976. Association of vesicular stomatitis virus proteins with HeLa cell membranes and released virus. J. Virol. **20**:637–645.
10. **Hunt, L. A., and D. F. Summers.** 1976. Glycosylation of vesicular stomatitis virus glycoprotein in virus-infected HeLa cells. J. Virol. **20**:646–657.
11. **Hunt, L. A., and D. F. Summers.** 1977. Glycosylation of VSV glycoprotein is similar in cystic fibrosis, heterozygous carrier, and normal human fibroblasts. J. Supramol. Struct. **7**:213–221.
12. **Krag, S. S., M. Difone, P. W. Robbins, and R. M. Baker.** 1977. Reduced synthesis of ^{14}C mannosyl oligosaccharide-lipid by membranes prepared from concanavalin A-resistant Chinese hamster ovary cells. J. Biol. Chem. **252**:3561–3564.
13. **Leavitt, R., S. Schlesinger, and S. Kornfeld.** 1977. Impaired intracellular migration and altered solubility of nonglycosylated glycoproteins of vesicular stomatitis virus and Sindbis virus. J. Biol. Chem. **252**:9018–9023.

14. **Li, E., I. Tabas, and S. Kornfeld.** 1978. The synthesis of complex-type oligosaccharides. I. Structure of the lipid-linked oligosaccharide precursor of the complex-type oligosaccharides of the vesicular stomatitis virus G protein. J. Biol. Chem. **253**:7762–7770.
15. **Moyer, S. A., J. M. Tsang, P. H. Atkinson, and D. F. Summers.** 1976. Oligosaccharide moieties of the glycoprotein of vesicular stomatitis virus. J. Virol. **18**:167–175.
16. **Narasimhan, S., P. Stanley, and H. Schachter.** 1977. The control of glycoprotein synthesis: a lectin-resistant mutant containing only one of two distinct N-acetylglucosaminyl-transferase activities present in wild type Chinese hamster ovary cells. J. Biol. Chem. **252**:3926–3933.
17. **Poles, P. G., and W. R. Gallaher.** 1979. Insensitivity of a ricin-resistant mutant of Chinese hamster ovary cells to fusion induced by Newcastle disease virus. J. Virol. **30**:69–75.
18. **Reading, C. L., E. E. Penhoet, and C. E. Ballou.** 1978. Carbohydrate structure of vesicular stomatitis virus glycoprotein. J. Biol. Chem. **253**:5600–5612.
19. **Robbins, P. W., S. C. Hubbard, S. J. Turco, and D. F. Wirth.** 1977. Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins. Cell **12**:893–900.
20. **Robertson, J. S., J. R. Etchison, and D. F. Summers.** 1976. Glycosylation sites of vesicular stomatitis virus glycoprotein. J. Virol. **19**:871–878.
21. **Robertson, M. A., J. R. Etchison, J. S. Robertson, D. F. Summers, and P. Stanley.** 1978. Specific changes in the oligosaccharide moieties of VSV grown in different lectin-resistant CHO cells. Cell **13**:515–526.
22. **Schlesinger, S., C. Gottlieb, P. Feil, N. Gelb, and S. Kornfeld.** 1976. Growth of enveloped RNA viruses in a line of Chinese hamster ovary cells with deficient N-acetyl-glucosaminyltransferase activity. J. Virol. **17**: 239–246.
23. **Sly, W. S., E. Lagwinska, and S. Schlesinger.** 1976. Enveloped virus acquires membrane defect when passaged in fibroblasts from I-cell disease patients. Proc. Natl. Acad. Sci. U.S.A. **73**:2443–2447.
24. **Stanley, P., V. Caillibot, and L. Siminovitch.** 1975. Selection and characterization of eight phenotypically distinct lines of lectin-resistant Chinese hamster ovary cells. Cell **6**:121–128.
25. **Stanley, P., S. Narasimhan, S. Siminovitch, and H. Schachter.** 1975. Chinese hamster ovary cells selected for resistance to the cytotoxicity of phytohemagglutinin are deficient in a UDP-N-acetylglucosamine-glycoprotein N-acetylglucosaminyltransferase activity. Proc. Natl. Acad. Sci. U.S.A. **72**:3323–3327.
26. **Stanley, P., and L. Siminovitch.** 1977. Complementation between mutants of CHO cells resistant to a variety of plant lectins. Somatic Cell Genet. **3**:391–405.
27. **Tabas, I., and S. Kornfeld.** 1978. The synthesis of complex-type oligosaccharides. III. Identification of an α-D-mannosidase activity involved in a late stage of processing of complex-type oligosaccharides. J. Biol. Chem. **253**:7779–7786.
28. **Tabas, I., S. Schlesinger, and S. Kornfeld.** 1978. Processing of high mannose oligosaccharides to form complex type oligosaccharides on the newly synthesized polypeptides of the vesicular stomatitis virus G protein and the IgG heavy chain. J. Biol. Chem. **253**:716–722.
29. **Tai, T., K. Yamashita, and A. Kobata.** 1977. The substrate specificities of endo-β-N-acetylglucosaminidase c_{II} and H. Biochem. Biophys. Res. Commun. **78**: 434–441.
30. **Tai, T., K. Yamashita, M. Ogata-Arakawa, N. Koide, T. Muramatsu, S. Iwashita, Y. Inque, and A. Kobata.** 1975. Structural studies of two ovalbumin glycopeptides in relation to the endo-β-N-acetylgocosaminidase specificity. J. Biol. Chem. **250**:8569–8575.
31. **Tarentino, A. L., and F. Maley.** 1974. Purification and properties of an endo-β-N-acetylglucosaminidase from *Streptomyces griseus*. J. Biol. Chem. **249**:811–817.
32. **Trowbridge, I. S., and R. Hyman.** 1979. Abnormal lipid-linked oligosaccharides in class E thy-1 negative mutant lymphomas. Cell **17**:503–508.
33. **Wagner, R. R.** 1975. Reproduction of rhabdoviruses, p. 1–93. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Publishing Corp., New York.