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Oligosaccharide Moieties of the Glycoprotein of Vesicular Stomatitis Virus

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Vesicular stomatitis virus contains a single structural glycoprotein whose carbohydrate sequences are probably specified by the host cell. The glycopeptides derived by Pronase digestion of the glycoprotein of vesicular stomatitis virus grown in HeLa cells have an average molecular weight of 1,800. There are multiple oligosaccharide chains on the vesicular stomatitis virus glycoprotein with protein-carbohydrate linkages that are cleaved only by strong alkali under reducing conditions, suggesting that they contain asparagine and *N*-acetylglucosamine. The oligosaccharide moieties, in addition, appear to be heterogeneous in sequence on the basis of their mobilities during electrophoresis and their sensitivities to cleavage by an endoglycosidase. The carbohydrate-peptide linkage region of the major class of oligosaccharides of the vesicular stomatitis virus glycoprotein has the proposed sequence:

$$\ldots (\text{Man})_n \rightarrow \text{GlcNAc} \rightarrow \text{GlcNAc} \rightarrow \text{Asn} \rightarrow (\text{Peptide})$$
$$\uparrow$$
$$\text{Fuc}$$

Vesicular stomatitis virus (VSV) is a membrane enveloped rhabdovirus (12) which contains a single-stranded RNA genome of 3.6×10^6 daltons (19) and five structural proteins (27). The nucleocapsid protein N, the protein NS, and the L protein, together with the RNA, form the ribonucleoprotein core, which is surrounded by the lipid envelope containing the membrane protein M and the single virus-specified glycoprotein G (27). In the infected cell the G protein becomes associated with the host cell plasma membrane during the process of virion maturation (3, 5) and constitutes the projections from the virus envelope in purified VSV (2, 16). The glycoprotein of VSV is the major virion antigen that induces and reacts with the VSV-neutralizing antibody (13). Moreover, in recent work it has been shown that the sialic acid residues of the G protein play a role in the infectivity of the virus (22) and that the VSV sialo-glycoprotein mediates the virus attachment to the cell surface (23).

Studies on the oligosaccharide moieties have shown that the purified VSV glycoprotein contains about 10% carbohydrate by weight with sugars normally found in plasma-type glycoproteins (8, 9, 24). The carbohydrate moieties of the glycoproteins of enveloped viruses are likely host specified (1, 10), and their size, composition (1, 9), and probably their sequence as well (18) vary in VSV grown in different cell lines. In this study we have examined some structural features of the oligosaccharide chains of the glycoprotein of VSV grown in HeLa cells to determine their number, size, carbohydrate-peptide linkage, and sequence heterogeneity. In partial characterization of the oligosaccharide sequences we have utilized an endoglycosidase enzyme described by Muramatsu (20) and Koide and Muramatsu (14).

MATERIALS AND METHODS

Cells and virus. Stock preparations of VSV (Indiana serotype) were grown in HeLa S_3 suspension cultures, purified, and assayed as previously described (11, 19). VSV-infected cells were labeled from 4 to 16 h postinfection as described previously (18) with radioactive sugars at the following final specific activities (per milliliter): 10 μCi of D-[6-^{3}H(N)]glucosamine hydrochloride (7.3 Ci/mmol); 10 μCi of L-[1,5,6-^{3}H]fucose (4.8 Ci/mmol) (obtained from New England Nuclear), and 10 μCi of D-[2-^{3}H]mannose (2.0 Ci/mmol) (obtained from Amersham Searle).

Preparation and purification of VSV glycopeptides. Purified radiolabeled samples (0.1 ml, 2 to 3 mg/ml) of VSV were dissociated by the addition of

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0.1% sodium dodecyl sulfate and heated at 100 C for 2 min. The virion proteins were diluted 10-fold and digested with 5 mg of Pronase (Calbiochem) in 1 ml of 1 M Tris-hydrochloride (pH 8.4)–0.01 M $CaCl_2$–0.135 M NaCl with a small amount of toluene for 24 h at 37 C. An additional 5 mg of Pronase was added, and the reaction was continued for a total of 48 h. The reaction mixture was heated at 100 C for 1 min to inactivate Pronase, and the following markers were added for chromatography: 0.2 mg of blue dextran (type 2000, Pharmacia), 2 mg of fucose (Sigma), and 1 mg of stachyose (B grade, Calbiochem; molecular weight, 666). The sample was applied to a column (0.9 by 110 cm) of Sephadex G-50 (fine) that was equilibrated and eluted with 0.05 M ammonium acetate buffer, pH 6.0 (18). Fractions of 1.25 ml were collected, samples were analyzed for radioactivity, and 50-μl samples were assayed by the phenol-sulfuric acid method (6) for the positions of the carbohydrate markers. No fucose- or mannose-labeled material was excluded from the column after Pronase digestion of the glycoprotein preparations, although material containing glucosamine (<20%) was excluded and not analyzed further. The single included peak of radioactive glycopeptides (18) was pooled and lyophilized.

Glycosidase digestion of the VSV glycopeptides. A partially purified glycosidase preparation from *Diplococcus pneumoniae* contained β-*N*-acetylglucosaminidase (0.5 units/mg of protein), β-galactosidase (0.05 units/mg of protein), and endo-β-*N*-acetylglucosaminidase-D (400 μg of protein) that released mannose-labeled oligosaccharides from glycopeptides as described (14, 20). The carbohydrate-labeled VSV glycopeptide preparations, purified by chromatography on Sephadex G-50, were incubated with this mixture of glycosidases and 50 μl of neuraminidase (*Vibrio cholerae*, 500 units/ml, General Biochemicals) in 0.5 ml of a solution containing 10 mM Tris-hydrochloride (pH 7.0), 15 mM NaCl, and 0.01 M NaN_3 at 37 C for 16 h. The enzymes were then inactivated by heating at 100 C for 2 min. Neuraminidase digestion of glycopeptides was performed as above in reaction mixtures without the other glycosidases.

Analysis of glycopeptides and the products of the glycosidase digestion by Sephadex G-25 column chromatography. Blue dextran (0.2 mg) and stachyose and fucose (1 mg each) were added to the glycosidase digest as markers, and the sample solution was applied to a column (0.9 by 140 cm) of Sephadex G-25 (fine) that was equilibrated and eluted with 50 mM ammonium acetate (pH 7.0) unless otherwise specified. Fractions (1.25 ml) were collected, and aliquots were analyzed by the phenol-sulfuric acid method (6) to determine the position of the standard markers stachyose and fucose. Other materials used to standardize a G-25 column so that approximate molecular weights could be determined on identical columns were: H-2 alloantigen glycopeptide, molecular weight approximately 3,300 (21; a gift from Stanley Nathenson, Albert Einstein College of Medicine, Bronx, N.Y.); unfractionated ovalbumin glycopeptides, molecular weight approximately 1,500 (21) and [^{14}C]acetyl-Asn-$(GlcNAc)_2$-$(Man)_5$, molecular weight 1,393, both generous gifts from Takashi Muramatsu, Kobe University School of Medicine, Kobe, Japan; the endo-β-*N*-acetylglucosaminidase-D glycopeptide product of the latter: ([^{14}C]acetyl-Asn-GlcNAc), molecular weight 379. Calf thyroglobulin unit A and unit B glycopeptides, molecular weight approximately 1,800 and 3,000, respectively (a generous gift from A. M. Adamany, Albert Einstein College of Medicine), were ^{3}H acetylated in the peptide moiety as described by Koide and Muramatsu (14) and also used as standards.

Trypsin digestion and analysis of the VSV glycoprotein. Purified G protein was obtained from carbohydrate-labeled VSV by the low salt-Triton X-100 method of Emerson and Wagner (7). Preparation of tryptic peptides of the isolated G protein was carried out as detailed previously (4). Radioactive tryptic peptides of the G protein were suspended in 0.05 M Tris-hydrochloride (pH 8.0), applied to a column (2 by 50 cm) of DEAE-Sephadex A25 (Pharmacia), and eluted with a 1-liter linear gradient from 0 to 0.35 M NaCl in 0.05 M Tris-hydrochloride (pH 8.0). The flow rate was maintained at about 50 ml/h by pumping. A sample (0.5 ml) of each column fraction was taken to assay for radioactivity in a liquid scintillation spectrometer. The peak fractions of peptides were pooled, lyophilized, suspended in 1 ml of distilled water, and desalted on columns (1 by 25 cm) of Sephadex G-25. The radioactive glycopeptides were again pooled, lyophilized, and analyzed by high-voltage paper electrophoresis.

Paper electrophoresis. High-voltage paper electrophoresis was carried out on 1.5-inch (ca. 3.8-cm)-wide strips of Whatman no. 1 paper at pH 1.9 (1.5 M formic acid) or at pH 6.5 (pyridine-acetic acid-water, 10:0.4:89.6) at 3,500 V for 3 h (37 V/cm). Strips were cut into fractions and analyzed for radioactivity as previously described (21).

Alkaline hydrolysis of [^{3}H]fucose-labeled VSV glycopeptides. (i) Mild alkali-borohydride treatment. Fucose-labeled VSV in 0.75 ml of water was treated with 0.5 M NaOH-0.5 M $NaBH_4$ under nitrogen at room temperature for 48 h and then neutralized on ice by glacial acetic acid. Labeled reaction product was desalted by Sephadex G-25 chromatography prior to further analysis.

Material treated with mild alkali (and subsequently Pronase), when subjected to electrophoresis at pH 1.9, appeared basic, and the bulk moved to the negative pole. At pH 6.5 the material was either neutral or slightly acidic. Thus these glycopeptide fragments so derived displayed an amphoteric nature consistent with the presence of amino acids.

(ii) Strong alkali-borohydride treatment. Fucose-labeled VSV glycopeptide in 0.5 ml of water was treated with 1.0 M NaOH-1.0 M $NaBH_4$ at 100 C for 6 h under nitrogen in a sealed tube and then neutralized on ice by glacial acetic acid. Labeled reaction product was desalted by Sephadex G-25 chromatography prior to further analysis. Fucose-labeled VSV glycopeptides so treated before or after Pronase or glycosidase digestion and subjected to electrophoresis at either pH 1.9 or 6.5 migrated to the negative pole and thus appeared basic, contrasting with the usual behavior of glycopeptides, where an ampho-

teric nature was observed under these conditions. Hence fucose-labeled VSV glycopeptides after strong alkali-sodium borohydride treatment no longer behaved as if the fucose label was bound to moieties containing amino acids. The basic behavior can be explained by the presence of glucosamine residues deacetylated by the alkaline hydrolysis (15, 25). Thus, the protein-carbohydrate linkage appeared to be broken by strong, but not by mild, alkali-borohydride treatment.

RESULTS

Analysis of the carbohydrate-labeled VSV glycopeptides. VSV was grown in suspension cultures of HeLa cells in the presence of either [^{3}H]mannose, [^{3}H]glucosamine, or [^{3}H]fucose to label the carbohydrate moieties of the virus glycoprotein G. The radiolabeled virus was isolated and purified, and analysis of samples of the VSV polypeptides by polyacrylamide gel electrophoresis showed that each labeled sugar was incorporated only into the single VSV glycoprotein G (data not shown). Each preparation was then dissociated with SDS and extensively digested with Pronase. The resulting glycopeptides were initially purified by chromatography on Sephadex G-50 and were then analyzed by Sephadex G-25 column chromatography. Figure 1A shows that the mannose-labeled material, which eluted as a homogeneous, included a peak with a molecular weight of approximately 1,800. Similar molecular weights were also found in glycopeptide preparations radiolabeled with fucose or glucosamine.

The Pronase-digested material from each labeled VSV preparation was shown to be glycopeptide in nature by its amphoteric characteristics during high-voltage paper electrophoresis at different pH values. The data for the fucose-labeled VSV glycopeptides are shown in Fig. 2. The material contained positively charged groups, presumably amino acids, since they migrated to the negative pole at pH 1.9 (Fig. 2C) but remained near the origin or moved to the positive pole at pH 6.5 (Fig. 2A). The multiple glycopeptides observed upon electrophoresis at each pH were initially attributed to the presence of variable numbers of sialic acid residues at the end of the oligosaccharide chains. However, this does not appear to be the case, since treatment with neuraminidase altered the relative amounts of each species but still showed multiple glycopeptide species upon electrophoresis (Fig. 2B and D). It is possible that neuraminidase digestion was incomplete; however, the electrophoretic behavior (Fig. 2) was more consistent with the presence of acidic amino acids in the peptide moiety. We postulate that this electrophoretic separation of species was due to differences in the number and/or sequence of the carbohydrate moieties and not to variability in the amino acid moieties in the remaining peptide, as will be discussed below.

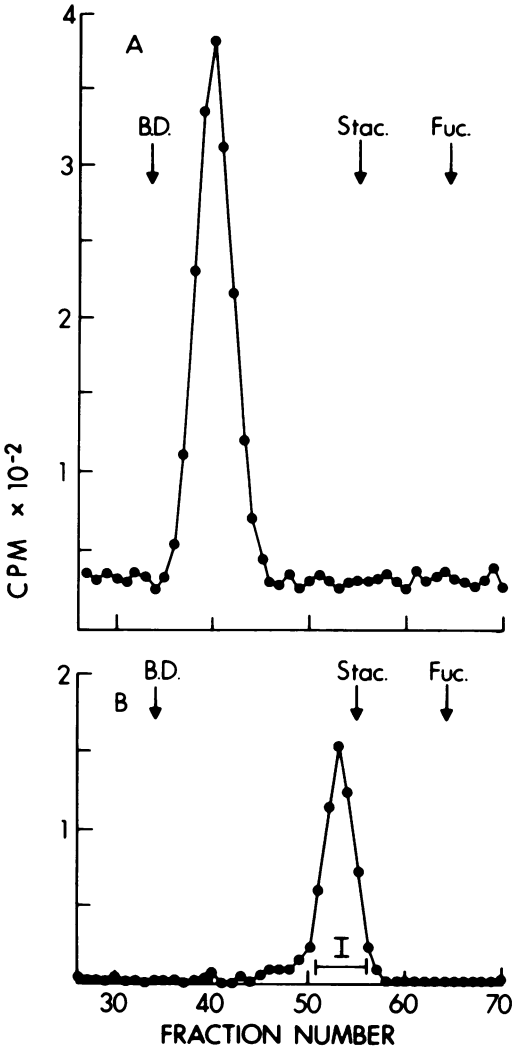


FIG. 1. *Sephadex G-25 column chromatography of mannose-labeled glycopeptides from VSV grown in HeLa cells. [^{3}H]mannose-labeled VSV was extensively digested with Pronase, and (A) a sample of the glycopeptides was directly analyzed by gel filtration on Sephadex G-25 as described. (B) Another sample of the glycopeptides was treated with the mixture of glycosidases, and the reaction products were chromatographed on Sephadex G-25. The fractions labeled I in (B) were pooled and lyophilized. The elution positions of the carbohydrate markers (blue dextran [BD], stachyose [Stac.], and fucose [Fuc.]) chromatographed with the samples are indicated by the arrows.*

To investigate the oligosaccharide sequences

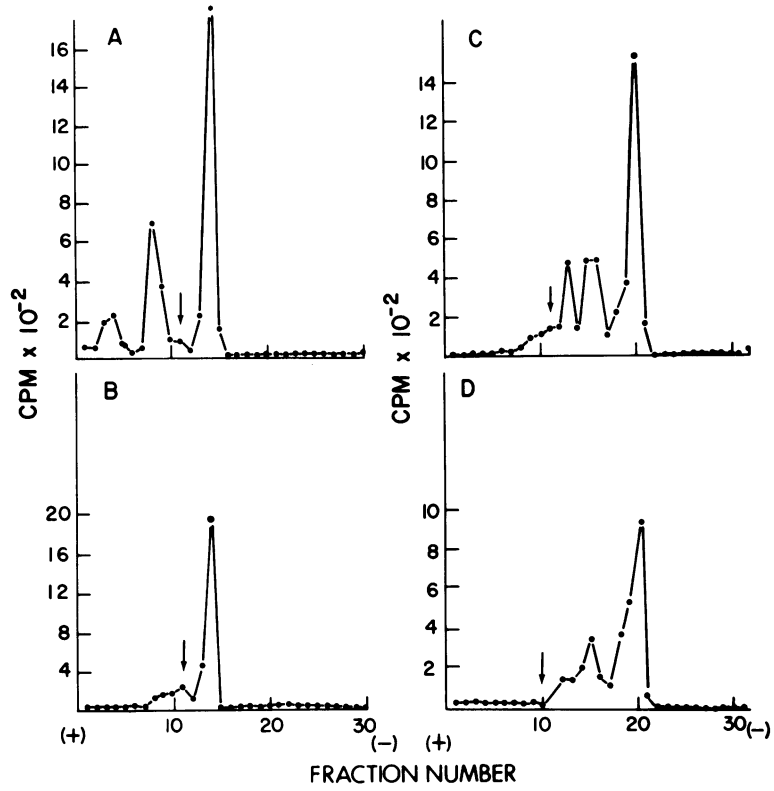


FIG. 2. *Paper electrophoretic analysis of the glycopeptides derived by Pronase digestion of fucose-labeled VSV. [³H]fucose-labeled VSV was digested with Pronase, and the glycopeptides were purified by chromatography on Sephadex G-50. The glycopeptides were pooled, lyophilized, and dissolved in water, and samples were analyzed by paper electrophoresis for 3 h at 3,500 V (A) in pyridine-acetic acid-water (pH 6.5) or (C) in 1.5 M formic acid (pH 1.9). Another sample was also digested iwth neuraminidase as described, and aliquots were analyzed (B) in pyridine-acetic acid-water (pH 6.5) or (D) in 1.5 M formic acid (pH 1.9). The paper was dried, cut into 1.5-cm strips, and counted. The origin at fraction 11 is indicated by the arrow.*

of these large glycopeptides in more detail, each purified preparation was further digested with a mixture of glycosidases, containing endo-β-*N*-acetylglucosaminidase-D (14, 20), β-*N*-acetylglucosaminidase, β-galactosidase, and neuraminidase enzymes as described above. These enzymes, in particular the endoglycosidase, have been useful for the study of the carbohydrate sequences in the human cell surface glycoproteins (21) and the glycoprotein of VSV grown in transformed and nontransformed BHK cells (18). The mannose-labeled glycopeptides were digested with the mixture of glycosidases and analyzed by chromatography on Sephadex G-25. Figure 1B shows the single product, the included material designated peak I, at a lower molecular weight than the undigested glycopeptide (Fig. 1A). This material has an apparent molecular weight of 460 when compared to glycopeptide standards. However, this estimate is certainly low since it has been shown that oligosaccharides chromatograph differently to glycopeptides on Sephadex G-25 (21); oligosaccharides are retarded less than glycopeptides of similar size. This fact is readily apparent in Fig. 1B, where glycopeptide standardization places peak I at a molecular weight of 460. However, peak I, being an oligosaccharide (neutral at pH 6.5 and 1.9 in HVPE; see Fig. 5B), must actually have a molecular weight greater than that of stachyose (666). Koide and Muramatsu (14) and also Tarentino and Maley (26) have shown that the endoglycosidase enzyme cleaves the di-*N*-acetylchitobiose structure [Asn-$(GlcNAc)_2(Man)_5$] in the ovalbumin glycopeptide between the two *N*-acetylglucosamine residues to release a glycopeptide fragment and a mannose-containing oligosaccharide. The endoglycosidase cleavage product observed for the mannose-labeled VSV glycopeptides would be consistent with the presence of this type of structure in VSV oligosaccharides.

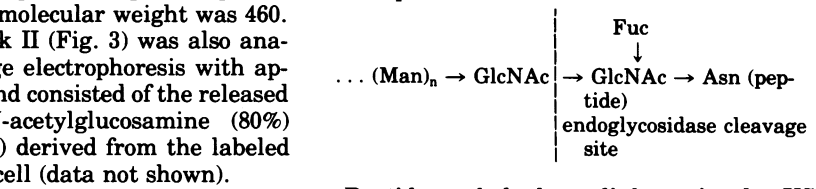
To determine some of the other carbohydrate

moieties that constitute the peptide-carbohydrate linkage region, the Pronase-digested VSV glycopeptides labeled with either glucosamine or fucose were also digested with the mixture of glycosidases containing the endoglycosidase-D. The products of these reactions were analyzed by chromatography on Sephadex G-25. Figure 3 shows that the [^{3}H]glucosamine-labeled products eluted as three included peaks. The largest component (fractions 38 to 44) had an approximate molecular weight of 1,600 and was probably a species that had lost terminal sialic acid residues and was insensitive to digestion by endo-β-*N*-acetylglucosaminidase-D. The material in peak I with an apparent molecular weight of 460 (Fig. 3) was shown to be a mixture of two species which were separable by electrophoresis (Fig. 5C). The latter consisted of a neutral glucosamine-labeled oligosaccharide (60%) that remained at the origin and, by the argument made above, must have a molecular weight greater than 460. There was also a positively charged glucosamine-labeled glycopeptide (30%) that migrated to the negative pole during electrophoresis at pH 1.9 whose molecular weight was 460. The material in peak II (Fig. 3) was also analyzed by high-voltage electrophoresis with appropriate markers and consisted of the released monosaccharides *N*-acetylglucosamine (80%) and sialic acid (20%) derived from the labeled glucosamine in the cell (data not shown).

In a similar reaction, the products of the glycosidase digestion of [^{3}H]fucose-labeled glycopeptides eluted as two glycopeptide peaks with approximate molecular weights of 1,600 and 460 (Fig. 4). The larger component (fractions 36 to 44), which was shown in the above experiment (Fig. 3) to contain glucosamine and now also fucose, appeared to be a glycopeptide resistant to digestion by endo-β-*N*-acetylglucosaminidase-D. The smaller reaction product, designated peak I, was shown to be entirely a glycopeptide fragment by its migration to the negative pole in electrophoresis at pH 1.9 (Fig. 5A). From these data and the known specificity of endo-β-*N*-acetylglucosaminidase-D (14, 26), a composition of X-Asn-GlcNAc-Fuc (where X is another amino acid) for the glycopeptide fragment would be consistent with its apparent molecular weight and carbohydrate composition.

The experiments utilizing the endoglycosidase enzyme as a probe to examine the structure of the oligosaccharides suggested that the VSV glycopeptides consisted of species with at least two different sequences. A portion of the glycopeptides were apparently not sensitive to endo-β-*N*-acetylglucosaminidase-D digestion and upon treatment with the mixture of glycosidases yielded a large glycopeptide (molecular weight, 1,600) that contained fucose and glucosamine but no mannose. This glycopeptide(s) must, therefore, have a different carbohydrate sequence than those species sensitive to endo-β-*N*-acetylglucosaminidase-D digestion.

The major products (peak I) produced by glycosidase digestion of the VSV glycopeptides from virus grown in HeLa cells consisted of a small glycopeptide fragment that contained glucosamine, fucose, and an amino acid(s) but no mannose and a released oligosaccharide of a similar molecular weight that contained glucosamine and mannose but no fucose. The small glycopeptide fragment presumably represents the few sugar and amino acid moieties that make up the carbohydrate linkage region of the VSV glycoprotein. Based on the known specificity of the reaction of the endo-β-*N*-acetylglucosaminidase-D enzyme with oligosaccharide moieties (14, 26) and the partial carbohydrate composition determined above, we propose that one class of the VSV glycopeptides has the partial sequence:



Peptide carbohydrate linkage in the VSV glycoprotein. The core glycopeptide (peak I) released by treatment of the 1,800-molecular-weight VSV glycopeptides with the mixture of glycosidases seemed to contain an amino acid, glucosamine, and fucose. The presence of glucosamine in this core material, together with data from other laboratories which showed that *N*-acetylgalactosamine was absent from (17) or present in very small amounts (8, 9) in the VSV glycoprotein, suggested that the protein-carbohydrate linkage may involve asparagine and *N*-acetylglucosamine rather than serine or threonine and *N*-acetylgalactosamine (24). To test this hypothesis, the fucose-labeled VSV glycoprotein and/or glycopeptides were hydrolyzed under conditions which specifically cleave only one of these types of linkages. Mild alkaline hydrolysis of glycoproteins has been shown to break only the serine- or threonine-*N*-acetylgalactosamine linkage (24), whereas strong alkaline digestion under reducing conditions is known to disrupt also the asparagine-*N*-acetylglucosamine linkage (15).

Intact fucose-labeled VSV was hydrolyzed under mild alkaline conditions by the procedure of Spiro (24) and subsequently digested

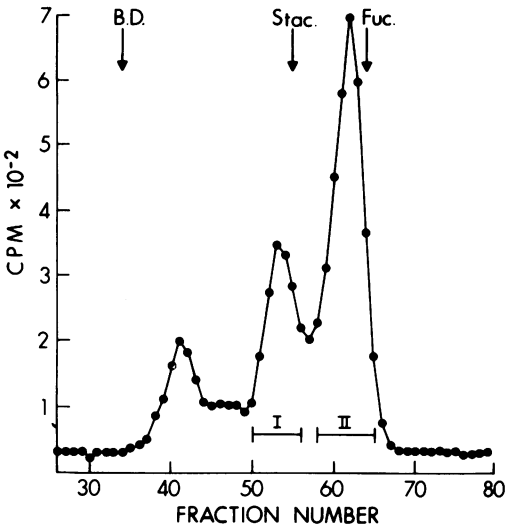


FIG. 3. *Sephadex G-25 column chromatography of the glycosidase-digested glycopeptides from glucosamine-labeled VSV. [³H]glucosamine-labeled VSV was digested with Pronase, and the glycopeptides were purified by chromatography on Sephadex G-50. The glycopeptides were digested with the mixture of glycosidases, and the reaction products were analyzed by Sephadex G-25 column chromatography with the carbohydrate markers indicated by the arrows as described in the legend to Fig. 1.*

with Pronase. Hydrolysis with mild alkali was carried out before Pronase digestion because the β-elimination reaction involved in the breakage of a possible serine or threonine-*N*-acetylgalactosamine linkage proceeds better if the glycosylated amino acid is not in a terminal position in the peptide chain (24). Hydrolysis of intact VSV guarantees that it would not be. The peptide-carbohydrate linkage was found to be insensitive to these digestion conditions, since the Pronase-digested product was a glycopeptide, as judged by high-voltage paper electrophoresis, and had a molecular weight of 1,800 in Sephadex G-25 column chromatography.

This glycopeptide was isolated and subsequently hydrolyzed under strong alkaline conditions (15, 24). This treatment released a fragment with an apparent molecular weight of 1,800 that behaved like a deacylated oligosaccharide on high-voltage electrophoresis at pH 6.5 and 1.9 (see above). These results are consistent with, but do not prove, a linkage group in the VSV glycoprotein of asparagine-*N*-acetylglucosamine.

Similarly, intact fucose-labeled VSV was digested with the glycosidase mixture as described previously. One portion was then directly digested with Pronase as a control, and another portion was hydrolyzed with strong alkali. Analysis of the glycosidase digestion products showed that the control, Pronase-treated material was a glycopeptide with a molecular weight of about 460 (cf. peak I, Fig. 4), whereas the released, hydrolyzed product was a deacylated oligosaccharide with an apparent molecular weight of 400, a result also consistent with the loss of an amino acid residue from the core glycopeptide after strong alkaline treatment.

To directly study the nature of the carbohydrate attachment site, we attempted to label the glycopeptides by growing VSV in the presence of radioactive aspartic acid. Possibly because of dilution of this label in cellular pools, or because extracellular aspartic acid is not the precursor of the asparagine in the glycoprotein, glycopeptides labeled with this precursor were never obtained. [³H]serine was also used to label VSV in the cell, and glycopeptides derived by extensive Pronase digestion of purified virus yielded ³H-labeled glycopeptides. This material was similar in size (~1,800 molecular weight) in Sephadex G-25 chromatography and in electrophoretic properties to carbohydrate-labeled glycopeptides. However, the label was not released by hydrolysis with mild alkali, as would

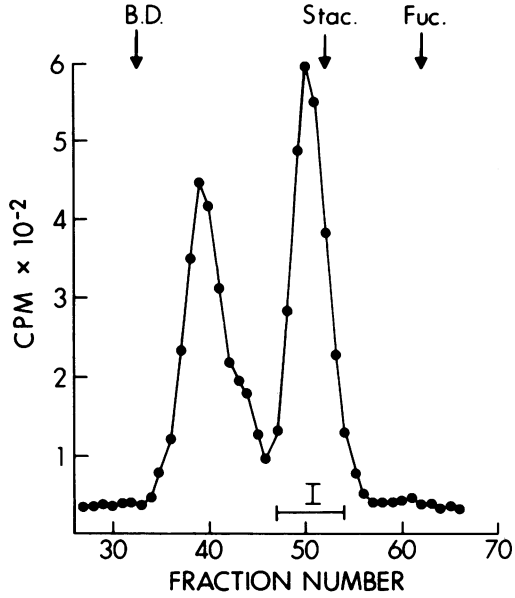


FIG. 4. *Sephadex G-25 column chromatography of the glycosidase-digested glycopeptides from fucose-labeled VSV. The glycopeptides from Pronase digestion of [³H]fucose-labeled VSV were purified by chromatography on Sephadex G-50, digested with the mixture of glycosidases, and analyzed by chromatography on Sephadex G-25 with the carbohydrate markers indicated by the arrows as described in the legend to Fig. 1.*

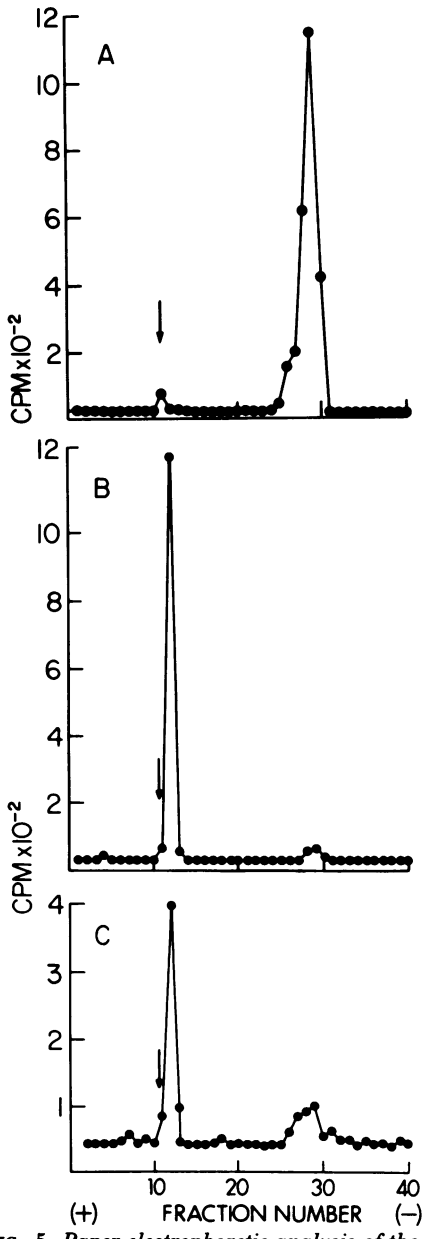


FIG. 5. *Paper electrophoretic analysis of the reaction products after glycosidase digestion of carbohydrate-labeled glycopeptides of VSV. The three labeled VSV glycopeptide preparations were digested with the mixture of glycosidases, and the reaction products were separated by chromatography on Sephadex G-25. The peak fractions eluting slightly faster than stachyose, designated I in Fig. 1B, 3, and 4, were pooled, lyophilized, dissolved in water, and analyzed by paper electrophoresis for 3 h at 3,500 V in 1.5 M formic acid (pH 1.9). The origin at fraction 11 is indicated by the arrow. Glycosidase digestion product I from (A) [³H]fucose-labeled glycopeptides, (B) [³H]mannose-labeled glycopeptides, and (C) [³H]glucosamine-labeled glycopeptides.*

have been expected if serine was the carbohydrate linkage amino acid. Label was released only upon treatment with strong alkali as low-molecular-weight material (probably serine) chromatographing in the amino acid region of the Sephadex G-25 column. We concluded from these experiments that serine was not the linkage amino acid but appeared instead to be a component of the peptide moiety of at least some of the glycopeptides.

Analysis of the tryptic peptides of the VSV glycoprotein. Since the above studies indicated that the oligosaccharide side chains of the VSV G protein are of at least two types, based on their reaction with the glycosidase mixture, we then attempted to directly determine the number of side chains per G molecule. VSV was grown in HeLa cells and labeled with [³H]glucosamine, and the glycoprotein was isolated from the purified virus by the procedure of Emerson and Wagner (7). After trypsin digestion of the ³H-labeled glycoprotein, the peptides were analyzed by chromatography on DEAE-Sephadex A25 as described above. Figure 6 shows that four major and several minor tryptic peptides contained ³H radioactivity derived from glucosamine. Each major peak was pooled, desalted, and further shown to be glycopeptide in nature by its amphoteric characteristics during high-voltage electrophoresis at pH 1.9 and 6.5 (data not shown).

DISCUSSION

In an effort to begin to characterize the structure of the VSV glycoprotein G, a study of the oligosaccharide moieties was undertaken. Preliminary investigations of this nature have previously been reported for VSV grown in BHK cells and a line of polyoma-transformed BHK cells (18). Since these studies indicated that the host cell in which the virus was grown altered the carbohydrate moieties of the G protein, the analysis of the glycoprotein was extended for comparison to virus grown in HeLa S_3 (spinner) cells. The glycopeptides of the G protein have several common features. Their size seemed to be homogeneous for a given preparation, although the glycopeptides from virus grown in HeLa S_3 cells have a smaller molecular weight (approximately 1,800) than those from virus grown in the BHK cell lines (3,000 [18]). Similar host-dependent size differences in glycopeptides have been reported for the glycoprotein of various enveloped viruses (1). In addition, two independent, although indirect, lines of evidence suggested that the oligosaccharide chains are joined to the G polypeptide backbone by asparagine-*N*-acetylglucosamine linkages. Firstly, endo-β-*N*-acetylglucosaminidase-D has a speci-

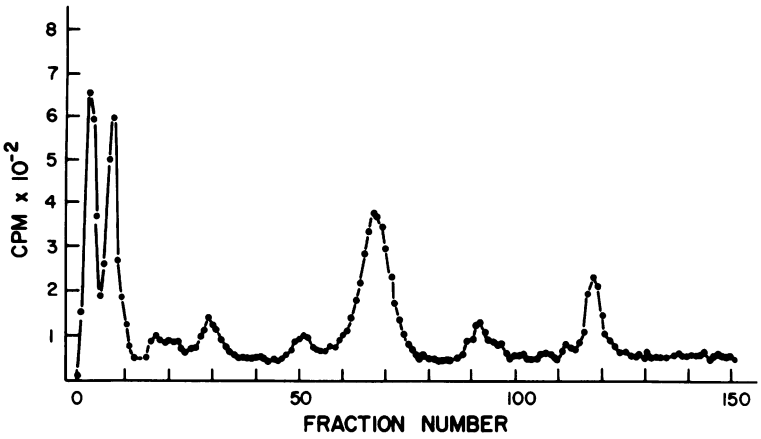


FIG. 6. *Analysis of the tryptic peptides of the glucosamine-labeled VSV glycoprotein by DEAE-Sephadex column chromatography. The [^{3}H]glucosamine-labeled glycoprotein was purified from labeled VSV and digested with trypsin as described. The tryptic peptides were analyzed by chromatography on a column of DEAE-Sephadex A25.*

ficity for the chemical components of this linkage, and, secondly, alkaline reduction tests also suggested this composition.

During our preliminary analysis of the fine structure of the oligosaccharides, however, it became evident that these moieties were heterogeneous in sequence. The VSV glycopeptides contained multiple species as shown by: (i) their differing electrophoretic mobilities during high-voltage electrophoresis; (ii) the presence of multiple glycosylated tryptic peptides of the G protein; and (iii) their differential sensitivities to digestion by endo-β-*N*-acetylglucosaminidase-D. In category (iii), we observed species labeled with mannose that were apparently completely digested by endo-β-*N*-acetylglucosaminidase-D. On the other hand, some species containing fucose and glucosamine were observed to be resistant to this enzyme, since their size was not much reduced after digestion. If mannose is indeed a general component of mammalian glycopeptides (27), then we must conclude that these resistant species are very minor components of the bulk of mannose-containing glycopeptides. Alternatively, such resistant species do not contain mannose. From these data the number of independently linked carbohydrate chains per glycoprotein molecule appeared to be not more than four. The appearance of at least two different sugar sequences does not in itself suggest at least two different linkage sites; different sequences might appear at that same linkage site in individual G molecules. Etchison and Holland (8, 9) have calculated that the VSV G protein derived from virus grown in a number of cell lines contained two glycopeptides with molecular weights of about 3,500. The resolution of the differences in the latter reports and the present communication must await the sequence analysis of oligosaccharides from the individual tryptic peptides by glycosidase treatment as outlined here for the total glycopeptide preparation. The partial structural analysis has so far suggested that there are two different carbohydrate sequences near the carbohydrate-peptide linkage region, but there may also be heterogeneity in the more distal region of the oligosaccharide chains.

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