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Carbohydrate Composition of the Membrane Glycoprotein of Vesicular Stomatitis Virus Grown in Four Mammalian Cell Lines

(enveloped RNA viruses/glycopeptides/glycoprotein glycosylation)

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ABSTRACT **The carbohydrate composition of the membrane glycoprotein of vesicular stomatitis virus has been determined for virus grown in four different mammalian cell lines. The glycoprotein contains mannose, galactose, *N*-acetylglucosamine, and neuraminic acid as the major carbohydrate components, whereas *N*-acetylgalactosamine and fucose are present in lesser amounts. The glycoprotein contains approximately 9–10% carbohydrate regardless of the host cell in which it is synthesized. Small quantitative differences are evident in the composition of the component sugars of the glycoprotein when the virus is grown in different host cells, and the glycoprotein of virus grown in a mouse fibroblast line (L cells) lacks fucose. The major oligosaccharide moieties of the virus glycoprotein from all cells are approximately the same size (3000–3400 daltons). The data presented here, in conjunction with previous data, indicate that the viral glycoprotein contains two major oligosaccharide constituents regardless of the host cell in which it is synthesized.**

Vesicular stomatitis virus (VSV) is an RNA-containing, enveloped virus that matures by budding through its host cell's plasma membrane (1, 2). The areas of plasma membrane where budding occurs, and the viral membrane envelope, contain proteins specified by the viral genome as well as lipids and glycolipids of cellular origin (3, 4). The envelope of VSV contains two protein species: a membrane matrix protein and a glycoprotein (which forms spike-like projections from the viral envelope).

The glycoprotein has an apparent molecular weight of 67,000 (5) and we show elsewhere that when the virus is grown in BHK21 cells, it contains two major oligosaccharide chains with molecular weights of 3000–3400 which represent approximately 10% of the glycoprotein by weight (6). Since the genome of VS virus is not sufficiently complex to specify the many glycosyl transferases which would be required to synthesize these oligosaccharide moieties, most of these enzymes, if not all, must be supplied by the host cell in which the virus replicates. Studies by Burge and Huang (7) indicate that the size of the oligosaccharide chains of purified VSV may differ when it is grown in Chinese hamster ovary cells rather than chick embryo fibroblasts. This difference was attributed to differences in sialic acid content. The relative distribution of the carbohydrate components of purified VSV grown in mouse fibroblasts has been reported to differ from that in chick embryo fibroblasts (8). The contributions of the virus glycolipid to these differences is not apparent, however, since these comparisons were not performed with the isolated glycoproteins.

Abbreviation: VSV, vesicular stomatitis virus.

The oligosaccharide moieties of glycoproteins in mammalian cells appear to be synthesized by the sequential addition of monosaccharides to the polypeptide structure. A battery of highly specific glycosyl transferases and compartmentalization of these enzymes are most likely instrumental in specifying the oligosaccharide structure (9). Several studies indicate that polypeptide structure may determine the glycosylation sites (10–13) and that the transferase which adds the first monosaccharide to these sites in the polypeptide may exhibit a stringent requirement for its polypeptide substrate (14). It is not known, however, to what extent, if any, the polypeptide structure may influence the oligosaccharide structure. It is known that the glucosyl transferase involved in collagen biosynthesis, which transfers glucose to galactose bound *O*-glycosidically to hydroxylsine, requires the free ϵ-amino group of the hydroxylysine residue (15). This indeed suggests that the influence of the polypeptide structure may extend beyond the biosynthesis of the initial carbohydrate-polypeptide linkage.

Since vesicular stomatitis virus has a broad host range, rapidly shuts off host macromolecular synthesis, and codes for the synthesis of a single glycoprotein polypeptide species, it provides an opportunity to study the glycosylation of a single polypeptide structure in several different biosynthetic environments. We have undertaken such a study and report here the carbohydrate composition of the VSV glycoprotein isolated from virus produced by four different species of mammalian cells in culture.

MATERIALS AND METHODS

Cell Culture. HeLa S3 cells (human cervical carcinoma), MDCK cells (Maden-Darby canine kidney epithelial cells), BHK21/C13 cells (baby hamster kidney fibroblasts), and L929 cells (mouse connective tissue fibroblasts) were grown as monolayers in Eagle's minimal essential medium supplemented with 5% heat-inactivated calf serum (30 min at 56°).

Virus. The Indiana serotype of vesicular stomatitis virus was used in these studies. Virus was prepared by infecting the cell monolayers with an effective multiplicity of 10–20 PFU (plaque-forming units) per cell and the growth medium was harvested 16 hr later. The virions were purified from the growth medium and the glycoprotein purified from the virions as described previously (6). [*6*-^{3}H]- and [*1*-^{14}C]Glucosamine labeled glycoprotein was prepared as described previously (6). Glycopeptides were prepared by digestion of the purified glycoprotein with 1 mg/ml of Pronase B (Calbiochem, San Diego) in 0.1 M Tris·HCl at pH 7.8, containing 3 mM $CaCl_2$ for 48 hr at 37°. Additional Pronase was added at 24 hr.

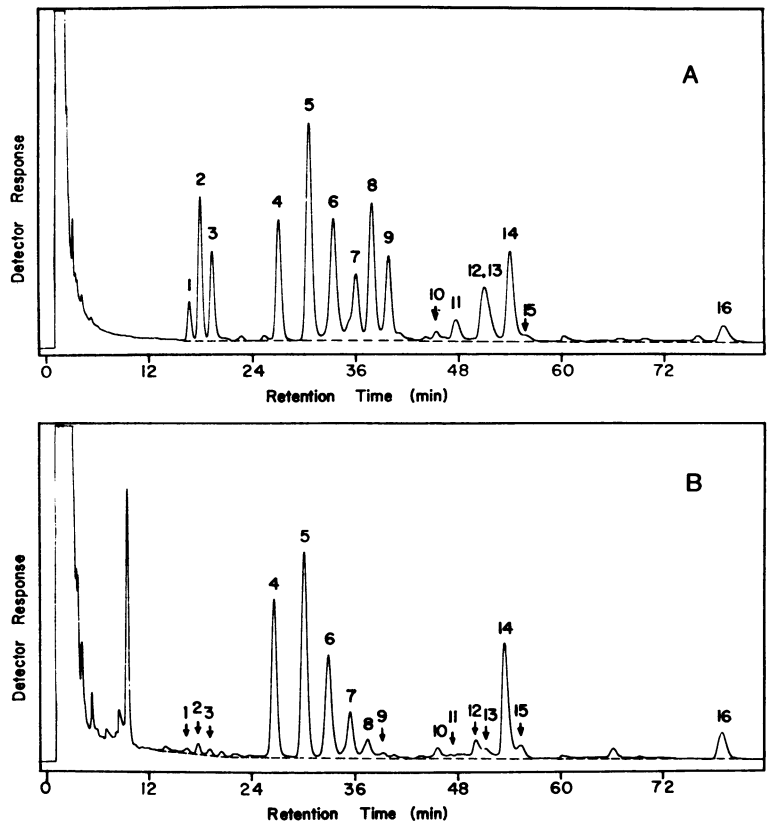

FIG. 1. Gas-liquid chromatography of the carbohydrate components of the membrane glycoprotein of vesicular stomatitis virus. (A) Analysis of a standard mixture of sugars. The identity of the peaks is as follows: fucose 1,2,3; arabitol 4; mannose 5; galactose 6,7; glucose 8,9; *N*-acetylgalactosamine 11,13; *N*-acetylglucosamine 10,12,14,15; *N*-acetylneuraminic acid (methyl ester) 16. (B) Chromatogram of the carbohydrates of the purified vesicular stomatitis virus glycoprotein from virus grown in HeLa cells (arabitol was added as an internal standard for quantitation purposes).

Carbohydrate Analysis. The carbohydrate composition of the purified glycoprotein was determined by gas-liquid chromatography of the trimethylsilyl ethers of the methyl glycosides (6, 16). Quantitation was performed using arabitol as an internal standard. Preparations containing 0.1–1.5 mg of the glycoprotein were methanolyzed with 0.5 ml of 1.5 N anhydrous methanolic HCl at 80° for 24 hr under N_2. After methanolysis, the amino sugars were re-*N*-acetylated by adding 0.15 ml of dry pyridine followed by 0.10 ml of acetic anhydride. The samples were thoroughly mixed after adding the acetic anhydride and incubated at room temperature for 1 hr (Etchison and Holland, in preparation). The samples were then taken to dryness *in vacuo* over KOH pellets and further dried *in vacuo* over P_2O_5 for at least 4 hr. The methyl glycosides were converted to their trimethylsilyl ethers by redissolving the samples in 50 μl of dry pyridine and adding 50 μl of *N,O*-bis(trimethylsilyl)-trifluoroacetamide (Regis Chemical Co., Morton Grove, Ill.). After a 30-min reaction at room temperature, the insoluble material was removed by centrifugation into the cone of a 1 ml Reacti-Vial (Pierce Chemical Co., Rockford, Ill.), and the supernatant taken to dryness with a stream of high purity dry N_2. The trimethylsilylated methyl glycosides were then redissolved in 15–50 μl of hexane and 1–3 μl injected into the gas chromatograph. Conditions of separation were as described previously (6).

Gel Filtration. Gel filtration analysis of glycopeptides was carried out on a 1 × 50-cm column of BioGel P-10 (Bio-Rad Laboratories, Richmond, Calif.). The flow rate was 10 ml/hr and 0.8-ml fractions were collected. Radiolabeled glycopeptides were located by liquid scintillation counting. The column was calibrated for molecular weight estimation using fetuin glycopeptides (17), ovalbumin glycopeptides (18), and neuraminlactose (Calbiochem, La Jolla, Calif.) as included markers of established molecular weight. Fetuin glycopeptides and neuraminlactose were located by hydrolyzing the fractions in 0.1 N H_2SO_4 for 60 min at 80° and then assaying released neuraminic acid by the thiobarbituric acid procedure of Warren (19). Ovalbumin glycopeptides were located by the phenol-sulfuric acid procedure (20). The excluded volume was located with Blue Dextran 2000 (Pharmacia Fine Chemicals, Piscataway, N.J.) and the totally included volume with [*1*-^{14}C]glucosamine.

RESULTS

The analysis of the carbohydrate constituents of the VSV glycoprotein by gas-liquid chromatography is typified by the chromatogram shown in Fig. 1. Panel A shows a mixture of the sugars commonly present in mammalian glycoproteins. Panel B shows the carbohydrate constituents of the VSV glycoprotein obtained from virus produced by infected HeLa cells. The individual peaks are identified in the figure legend. The major carbohydrate components of the glycoprotein are clearly mannose, galactose, *N*-acetylglucosamine, and neuraminic acid. Fucose and *N*-acetylgalactosamine are present

TABLE 1. *Carbohydrate composition of the glycoprotein of VSV grown in different host cells**

	Host cell in which virus was grown			
	BHK21 (8)†	HeLa (4)†	L929 (4)†	MDCK (2)†
Fucose	0.28 ± 0.08	0.21 ± 0.04	≤0.04	0.45
Man	1.97 ± 0.29	2.15 ± 0.09	2.03 ± 0.69	2.04
Gal	1.61 ± 0.19	1.41 ± 0.13	1.64 ± 0.20	1.68
GlcNAc	3.31 ± 0.37	3.36 ± 0.15	2.51 ± 0.27	2.84
NeuNAc	2.27 ± 0.72	2.14 ± 0.39	2.28 ± 0.33	2.21
GalNAc	0.42 ± 0.17	0.37 ± 0.12	0.22 ± 0.06	0.61
Total	9.85 ± 1.82	9.64 ± 0.92	8.72 ± 1.59	9.83

* Expressed as μg/100 μg of the purified glycoprotein which had been dialyzed against distilled water, lyophilized, and dried *in vacuo* for 24 hr.

† The number in parentheses indicates the number of different preparations analyzed. The values below represent the arithmetic mean and standard deviation from the mean.

TABLE 2. *Carbohydrate residues per glycoprotein of VSV grown in different host cells**

	Host cell in which virus was grown			
	BHK21	HeLa	L929	MDCK
Fucose	1.21 ± 0.12	0.85 ± 0.09	≤0.12	1.83
Man	7.31 ± 0.38	8.02 ± 0.19	7.57 ± 1.2	7.61
Gal	5.99 ± 0.25	5.25 ± 0.27	6.11 ± 0.37	6.26
GlcNAc	10.1 ± 0.45	10.2 ± 0.26	7.62 ± 0.41	8.62
NeuNAc	4.93 ± 0.55	4.65 ± 0.49	4.96 ± 0.36	4.81
GalNAc	1.27 ± 0.10	1.12 ± 0.29	0.67 ± 0.10	1.85

* The numbers given here were computed from the mean values given in Table 1 using a molecular weight of 67,000 for the glycoprotein (5). Where possible, the standard error of the mean is also shown.

in lesser amounts. Glucose was present in variable amounts in most preparations and is most likely due to cellulosic contamination; some preparations contained very little (6).

The results of quantitative analysis of the carbohydrate of the VSV glycoprotein from virions produced in four different mammalian cell cultures is shown in Tables 1 and 2. The glycoprotein from all four sources contains approximately 9–10% carbohydrate. The distribution of this carbohydrate among the component sugars is quite similar overall. Some differences, however, are evident. The glycoprotein from VSV grown in L929 cells lacks fucose and contains less *N*-acetylglucosamine. The glycoprotein from MDCK-grown VSV contains more fucose and *N*-acetylgalactosamine than the glycoproteins from the other sources. The carbohydrate composition of the glycoprotein from virions grown in BHK21 and HeLa cells is essentially the same. These results demonstrate that both qualitative and quantitative differences may occur, although the basic composition is similar in all cases. These differences and similarities are most readily apparent in Table 2, which shows the carbohydrate composition as residues per glycoprotein.

Fig. 2 shows a comparison of the size of the glycopeptides of the glycoproteins from the four different cell lines. [*1*-^{14}C]-$GlcNH_2$ labeled VSV glycoprotein obtained from BHK21 grown virus was mixed with [*6*-^{3}H]$GlcNH_2$ labeled VSV glycoprotein obtained from virus that had been grown in each of the other three cell lines, and the mixtures were exhaustively digested with Pronase. The resulting glycopeptides were sized by gel filtration on BioGel P-10. As can be seen, the glycopeptides are approximately the same size regardless of the cell line in which the virus is grown. By comparing the relative elution of the VSV glycopeptides to the glycopeptides of fetuin and ovalbumin, and assuming that the glycopeptides contained approximately 10% amino acids, i.e., 2 or 3 amino-acid residues per glycopeptide, the molecular weight of the oligosaccharides of the VSV glycoprotein is estimated to be in the range of 3000–3400. Since the molecular weight of the glycoprotein is approximately 67,000 (5) and it contains 10% carbohydrate by weight, it follows that there are two such oligosaccharides per glycoprotein.

The glycopeptides from HeLa- and MDCK-grown VSV glycoprotein contain a minor component which chromatographs as a heterogeneous shoulder on the large molecular weight side of the major glycopeptide component. It is not yet clear whether this represents the presence of some larger oligosaccharide material or whether a difference in the carbohydrate core structure, proximal to the peptide moiety, may render the polypeptide more resistant to Pronase digestion near the carbohydrate-polypeptide junction.

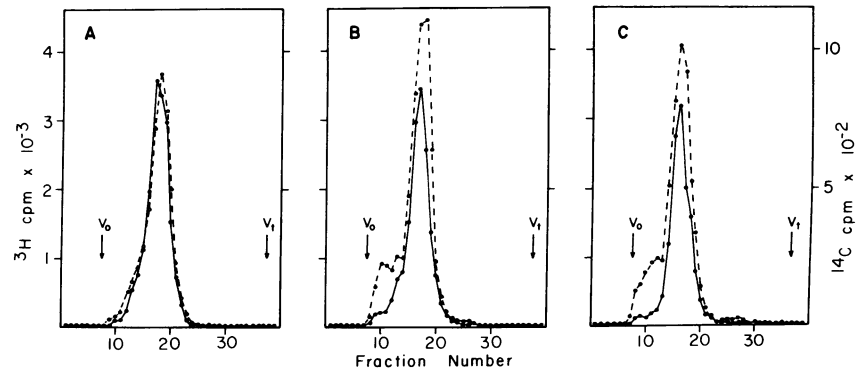

FIG. 2. Comparison of VSV glycopeptides by gel filtration. Purified [^{3}H]$GlcNH_2$ labeled glycoproteins from virus grown in L929, HeLa, and MDCK cells were mixed with [^{14}C]$GlcNH_2$ labeled glycoprotein from BHK21-grown virus. The mixtures were digested with Pronase and chromatographed on a column of BioGel P-10. (A) L929-VSV glycopeptides versus BHK21-VSV glycopeptides, (B) HeLa-VSV glycopeptides versus BHK21-VSV glycopeptides, and (C) MDCK-VSV glycopeptides versus BHK21-VSV glycopeptides. (O– –O is ^{3}H; ●——● is ^{14}C). The arrows mark the column void volume (V_o) and the totally included volume (V_t).

DISCUSSION

The results presented here and elsewhere (6) allow us to conclude the following: (*a*) both qualitative and quantitative differences occur in the carbohydrate composition of the VSV glycoprotein when it is synthesized in different host cells, and (*b*) there are two major oligosaccharide moieties with molecular weights of 3000–3400 attached to the VSV glycoprotein in all four cell lines examined. While the differences have been pointed out, it is also important to note that, in the four cell lines studied here, the overall composition is quite similar. As noted previously (6) we have not excluded the possibility of the presence of minor, low-molecular-weight glycopeptides which do not contain glucosamine.

The absence of fucose and the decreased amount of *N*-acetylglucosamine in the VSV glycoprotein synthesized in L929 cells as compared to the other cell lines, suggests that the oligosaccharides of the glycoprotein may have a smaller core structure. The fucose residue present in the VSV glycoprotein obtained from BHK21 cells is attached to an *N*-acetylglucosamine residue proximal to the polypeptide (21) with a linkage similar to that in an oligosaccharide of γG myeloma protein (22).

The presence of qualitative and quantitative differences in the oligosaccharide moieties of the VSV glycoprotein when grown in different cells indicates that the polypeptide structure does not necessarily exert complete control over the entire structure of the oligosaccharide moiety. However, the overall similarity of the carbohydrate compositions and the size of the oligosaccharides may reflect a nonstringent influence of the polypeptide on the oligosaccharide structure.

The presence of two major oligosaccharides in all cases is especially interesting, since the glycosyl transferases involved in the synthesis of the carbohydrate–polypeptide junction must ascertain "glycosylation sites" in the polypeptide structure. Since this enzyme is likely to be involved in the regulatory mechanism committing a polypeptide to be a glycoprotein, one would expect it to impose a restrictive substrate requirement on the polypeptide. The presence of the same number of oligosaccharide moieties on the VSV glycoprotein in four different cell lines suggests that we may find the same sites glycosylated. If this is indeed the case, we would conclude that either the virus genome codes for the synthesis of this enzyme, or the enzyme(s) involved, is common to all four cell lines. No animal, plant, nor insect virus has yet been shown to code for the synthesis of a glycosyl transferase. It seems unlikely that a virus with a limited genome, such as VSV could afford to specify such an enzyme, but the information presently available does not allow us to exclude that possibility.

It is possible that membrane viruses such as VSV have evolved so as to acquire oligosaccharide chains of an ubiquitous type, e.g., serum glycoprotein type, common to many cells, rather than acquiring more specialized and variable chains (such as blood group oligosaccharides). It should be noted that the cells employed here are from diverse tissue sources as well as from diverse species, i.e., mouse fibroblast, dog kidney epithelium, hamster fibroblast, and human cervical carcinoma (epithelium).

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