JOURNAL OF VIROLOGY, Mar. 1971, p. 412-415
Vol. 7, No. 3
Copyright © 1971 American Society for Microbiology
Printed in U.S.A.

Carbohydrate Composition of Vesicular Stomatitis Virus

JAMES J. McSHARRY[1] AND ROBERT R. WAGNER

Department of Microbiology, The University of Virginia School of Medicine, Charlottesville, Virginia 22901

Received for publication 30 November 1970

Analysis by gas-liquid chromatography of the trimethylsilylated sugar residues of purified vesicular stomatitis virus grown in L cells or chick embryo cells revealed the presence in the whole virion of four hexoses (glucose, galactose, mannose, and fucose), two hexosamines (glucosamine and galactosamine), and 34 to 40% neuraminic acid. The isolated viral glycoprotein was devoid of galactosamine and fucose, both of which sugars were present in whole virions presumably as part of the membrane glycolipids.

In a previous report (6), we showed that the Indiana serotype of vesicular stomatitis (VS) virus is composed of approximately 3% ribonucleic acid, 65% protein, 20% lipid, and 13% carbohydrate (exclusive of pentose in ribonucleic acid). Chemical analysis of the nonpentose carbohydrate extracted from purified VS virions revealed about 30% neutral sugars, 40% hexosamines, and 30% sialic acid (6). One of the three major virion structural proteins has been shown to be a glycoprotein which incorporates radioactive glucosamine (1, 9). In this preliminary report, we describe results of quantitative analysis of the sugar residues of VS virus and its isolated glycopeptide by the method of gas-liquid chromatography.

VS virus (Indiana serotype) was propagated in L cells or primary chick embryo (CE) cells, as previously described (6, 9). Released virions were concentrated by precipitation with polyethylene glycol and further purified at least 150-fold by sequential chromatography, rate zonal centrifugation, and equilibrium centrifugation (6). Yields from 10^9 cells were approximately 10^{12} virions, representing about 5 mg of protein. Sucrose was removed after the final purification step by exhaustive dialysis against distilled water. Methylglycosides of the carbohydrates of the purified virions were prepared by reaction with anhydrous methanolic-hydrochloride by the method of Reinhold et al. (7); trimethylsilyl derivatives were then prepared by reaction with a mixture of hexamethyldisilazane and trimethylchlorosilane essentially by the procedures described by Carver and Graves (3) and analyzed in parallel with appropriate standards in a Beckman GC-4 gas chromatograph.

Table 1 compares the relative concentrations of sugar residues extracted from purified VS virions grown in L cells or CE cells. These results obtained by gas-liquid chromatography are in reasonably good agreement with the chemical analyses previously reported (6). The relative proportions of individual sugars are probably not significantly different for VS virions grown in L cells and CE cells, with the possible exception of galactose. Neuraminic acid was always the major sugar on repeated analyses. Both hexosamines constituted a large proportion of virion carbohydrate, whereas glucose was the only major neutral sugar compared with the considerably lesser amounts of fucose, mannose, and galactose. Similar distributions of neutral sugars, hexosamines, and neuraminic acid were noted in carbohydrate extracted from the New Jersey serotype (VS_{NJ}) of VS virus grown in L cells and CE cells. Figure 1 shows representative gas chromatographic tracings of silylated methylglycosides extracted from L cell-grown purified virions of VS_{Ind} and VS_{NJ} virus compared with sugar standards.

The preceding analyses of carbohydrates extracted from whole virions provide no basis for location of the various sugars. In addition to carbohydrates covalently bonded to the virion glycoprotein (1), the glycolipids present in the virion membrane undoubtedly account for a large proportion of the viral sugars (4). To identify the sugar residues specific for the glycoprotein, we used a modification of the method adapted by Burge and Strauss (2) to isolate and purify the glycopeptide component(s)

[1] Present address: Rockefeller University, New York, N.Y. 10021.

Downloaded from http://jvi.asm.org/ on September 28, 2020 by guest

TABLE 1. *Sugar content of purified VS virus and its isolated glycoprotein determined by gas-liquid chromatography*

Sugar[a]	Area of each sugar peak as per cent of total		
	L cell-grown virions[b]	CE-grown virions[b]	Glycoprotein[c]
Fucose	2.3	2.5	<0.1
Mannose	2.9	3.4	4.6
Galactose	3.6	0.9	5.4
Glucose	17.4	22.6	21.3
Galactosamine	19.0	13.7	<0.1
Glucosamine	20.6	17.0	31.1
Neuraminic acid	34.3	39.9	37.3

[a] Sugars are listed in increasing order of retention time on a 6 ft by ⅛ inch stainless steel column packed with chromsorb W coated with 3% dimethylsilicone gum polymer SE-30 (Applied Science Laboratories, State College, Pa.). The amino sugars were *N*-acetylated with acetic anhydride. The column was calibrated and retention times were calculated for trimethylsilyl derivatives of the methylglycosides of standards of ribose, fucose, mannose, galactose, glucose, *N*-acetylgalactosamine, *N*-acetylglucosamine, and *N*-acetylneuraminic acid. Samples eluting from the column were detected with a dual flame ionizer and recorded on a Beckman linear-log recorder. Good separation was achieved with a programmed temperature rise of 2 C per minute from 130 C isothermal period to 200 C. The relative concentrations of each sugar are recorded as the area under each peak with the total area considered as 100%. These results have not been corrected for mass or ion density of each sugar.

[b] VS virions were grown in L or CE cells and extensively purified and their carbohydrates were hydrolyzed in 0.5 N methanolic-hydrochloride for 12 hr before converting them to methylglycosides and trimethylsilyl derivatives. Ribose of VS viral ribonucleic acid was not readily hydrolyzed; only trace amounts of ribose were detected and are not considered in the calculations.

[c] Viral glycoprotein labeled with ^{3}H-glucosamine was extracted from L cell-grown purified VS virions with acetic acid, urea, sodium dodecyl sulfate, and 2-mercaptoethanol (9), separated by electrophoresis on a 7.5% polyacrylamide gel (Fig. 2), digested with Pronase, and chromatographed on Biogel P-6 and Biogel P-10. The peak fractions of ^{3}H-glycoprotein shown in Fig. 3 were hydrolyzed, and the trimethylsilyl derivatives were analyzed by gas chromatography.

of VS virions (1). Virus was grown in L cells by a method previously described (9) in medium containing D-[*1*-^{3}*H*]-glucosamine (1.3 Ci/mmole, Schwarz BioResearch) and reconstituted uniformly labeled ^{14}C-amino acids (54 mCi/matom, New England Nuclear Corp.). In a companion experiment the virus was grown in the presence of D-[*1*-^{3}*H*]-galactose (5.1 mCi/mmole, Schwarz BioResearch). Released virions were purified by sequential differential, rate zonal (sucrose), and equilibrium (CsCl) centrifugation. The proteins of the purified virions were then extracted and analyzed by electrophoresis on neutral polyacrylamide gels in the presence of urea and sodium dodecyl sulfate (SDS) as previously described (9).

Figure 2 demonstrates that ^{3}H-glucosamine was incorporated specifically into glycoprotein

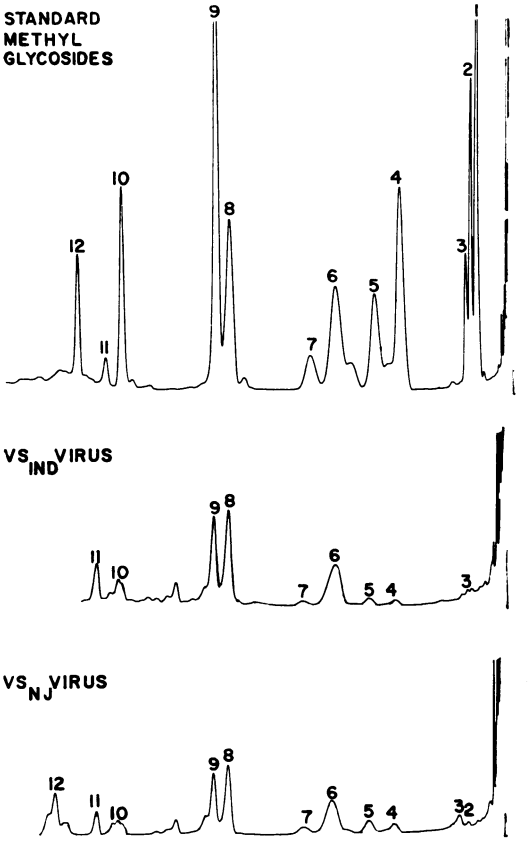

FIG. 1. *Gas-liquid chromatograms of silylated methylglycosides of a standard mixture of sugars compared with derivatives of sugars extracted from purified VS*$_{Ind}$ *and VS*$_{NJ}$ *virions propagated in L cells. Details of the procedure are summarized in the footnotes to Table 1. The numbered peaks represent the trimethylsilyl derivatives of methylglycosides of (1) ribose, (2, 3) fucose, (4) mannose, (5) galactose, (6, 7) glucose, (8) N-acetylgalactosamine, (9) N-acetylglucosamine, and (10, 11, 12) N-acetylneuraminic acid. Peaks representing lipid components are not shown; the lipids were retained longer than the sugar derivatives.*

Downloaded from http://jvi.asm.org/ on September 28, 2020 by guest

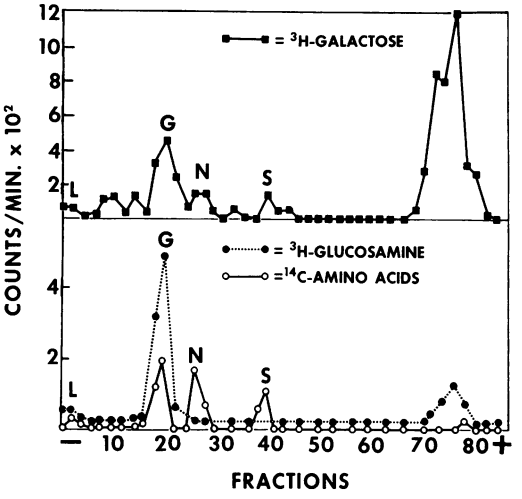

FIG. 2. *Electropherograms of the glycoprotein and presumed glycolipids of VS virus. L cell monolayers were infected with VS virus at a multiplicity of 10 and incubated in serum-free basal medium, Eagle's diluted 1:50 in Earle's saline solution and containing either ³H-galactose (5 μCi/ml) or ³H-glucosamine (1 μCi/ml) plus ¹⁴C-amino acids (1.13 μCi/ml). After 8 hr at 37 C, 5 ml of complete medium plus 2% calf serum was added to each culture. The medium was harvested at 17 hr after infection, and virions were concentrated and purified. Viral proteins were solubilized by treatment with a 0.1 volume of glacial acetic acid, 0.5 M urea, and 1% SDS at 37 C for 1 hr followed by overnight dialysis against 1,000 volumes of 0.01 M phosphate buffer, pH 7.2, containing 0.1% SDS, 0.5 M urea, and 0.1% 2-mercaptoethanol. Electrophoresis was carried out at 5 ma/gel for 6 hr on 7.5% polyacrylamide gels (10 cm in length) in the presence of 0.1 M phosphate buffer (pH 7.2) containing 0.5 M urea and 0.1% SDS. Protein bands stained with Coomassie blue were correlated with peaks of radioactivity in gels sliced into lengths of 1.25 mm as previously described (9). The letters designate the migration of the large protein (L), glycoprotein (G), nucleoprotein (N), and surface protein (S). The large amount of ³H that migrated to the anode is presumed to be incorporated into glycolipids.*

(G) and not into proteins L, N, and S. ³H-galactose was also incorporated into the G protein of VS virus, but in this case, lesser amounts of label were noted in the regions of the other virion proteins. In addition, a considerable amount of ³H-galactose and ³H-glucosamine label migrated far down the gel to a region devoid of labeled proteins; this activity is consistent with the behavior of glycolipids on polyacrylamide gels electrophoresed under these conditions (8).

Seven gels identical to, and run simultaneously with, the one shown in the lower panel of Fig. 2 were stained with Coomassie blue and the isolated stained glycoprotein (G) bands were excised with a razor blade. These 7 gel fragments were pooled and homogenized with a glass rod in 1 ml of 0.1 M sodium phosphate buffer (*p*H 7.8) containing 0.002 M $CaCl_2$; the glycoprotein was degraded to glycopeptide by incubation at 37 C with 0.1% Pronase (Calbiochem, Los Angeles) added each day for 4 days. The Pronase was autodigested at 37 C for 1 hr before use to minimize possible glycosylase activity. The digested ³H-glycopeptide fraction eluted from the gel fragments was dialyzed against distilled water and chromatographed on a Biogel P-6 column equilibrated with phosphate-buffered saline containing 0.1% SDS, as described by Burge and Strauss (2). The ³H-glycopeptide fraction was eluted from the Biogel P-6 column as a single sharp peak just behind an excluded dye, blue dextran 2000. The peak fractions of ³H radioactivity were pooled, concentrated by evaporation, and rechromatographed on a Biogel P-10 column.

Figure 3 shows the elution pattern of the VS

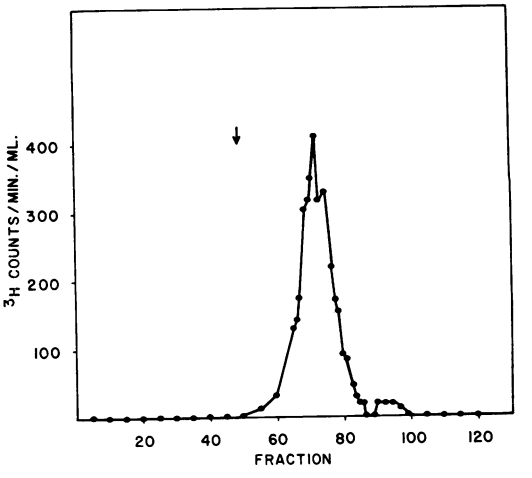

FIG. 3. *Exclusion chromatography on Biogel P-10 of purified ³H-glycopeptide of VS virus. Proteins extracted from virions grown in the presence of ³H-glucosamine and ¹⁴C-amino acids were electrophoresed in a manner identical to that shown in the lower panel of Fig. 2. The glycoprotein bands from 7 gels stained with Coomassie blue were excised, homogenized, digested for 4 days with Pronase and chromatographed on a Biogel P-6 column. The peak fractions of ³H-glycopeptide from the P-6 Biogel column were chromatographed on a column (0.9 by 56 cm) of Biogel P-10 in 0.1 M phosphate buffer (pH 7.2) containing 0.1% SDS. The flow rate was 6 ml per hour, and fractions of 0.5 ml each were collected. The exclusion volume and void volume of the column were measured with blue dextran 2000 (arrow) and potassium dichromate. Fetuin glycopeptide (not run simultaneously) was eluted in the same peak region as the ³H-glycopeptide of VS virus.*

Downloaded from http://jvi.asm.org/ on September 28, 2020 by guest

virus glycopeptide labeled with ^{3}H-glucosamine. No ^{14}C counts could be detected in the eluate from the P-6 or the P-10 columns, indicating that Pronase had digested virtually all of the protein component of the glycoprotein to dialyzable oligopeptides or amino acids. Recovery of ^{3}H counts was virtually 100%, and the position of the radioactive peak shown in Fig. 3 is essentially the same as that described by Burge and Huang (1); elution of fetuin glycopeptide from our Biogel P-10 column confirmed a molecular weight of ~4,000 daltons for the VS virus glycopeptide reported by Burge and Huang (1). Except for a minor trailing peak on Biogel P-10 (Fig. 3), not observed on Biogel P-6, the glycopeptide of VS virus appears to be relatively homogeneous.

The peak fractions of radioactivity eluted from the Biogel P-10 column were pooled, dialyzed against distilled water and concentrated by evaporation; trimethylsilyl derivatives of methylglycosides were prepared and analyzed by gas-liquid chromatography. Table 1 shows the results of the sugar analysis of the isolated viral glycopeptide. As noted, neuraminic acid was the major component of the glycopeptide as well as the whole virion, which also contained similar proportions of mannose, galactose, glucose, and glucosamine. However, in striking contrast to the carbohydrate composition of the whole virion, the viral glycopeptide contained no detectable galactosamine or fucose.

We have not yet analyzed the sugar content of the VS virion glycolipids, but the electropherograms (Fig. 2) strongly suggest the presence of low-molecular-weight glycolipids, containing galactose and glucosamine, which are readily dissociated from the viral glycoprotein. Preliminary data also reveal incorporation of ^{14}C-galactosamine into a rapidly migrating glycolipid component but not the glycoprotein (Wagner, *unpublished data*). The glycolipid of the VS virion envelope is almost undoubtedly derived from cellular plasma membrane, as indicated by the experiments of Klenk and Choppin (5), who found that VS virions grown in BHK-F cells contained the same hematoside, neuraminosyl-galactosyl-glucosyl-ceramide, as did the host cells. About one-third of the carbohydrate content of the enveloped SV5 virion was found to be present as glycolipid and is undoubtedly derived from the host cell (4). The hypothesis proposed by Burge and Huang (1) that the carbohydrate moieties of Sindbis and VS viral glycoproteins are specified by the host cell seems reasonable but was not confirmed by the data of Klenk et al. (4) on SV5 glycoproteins grown in different cells. Analysis of sugar content of viral glycoproteins and glycolipids by gas-liquid chromatography should settle many of the questions concerning their origin.

We acknowledge the excellent technical assistance of Nancy Salomonsky.

This research was supported by Public Health Service grant CA-10387 from the National Cancer Institute and grant GB-6537X from the National Science Foundation.

LITERATURE CITED

1. Burge, B. W., and A. S. Huang. 1970. Comparison of membrane protein glycopeptides of Sindbis virus and vesicular stomatitis virus. J. Virol. 6:176–182.
2. Burge, B. W., and J. H. Strauss, Jr. 1970. Glycopeptides of the membrane glycoprotein of Sindbis virus. J. Mol. Biol. 47:449–466.
3. Carver, H. E., and R. C. Graves. 1967. Improved reagent for trimethylsilylation of sphingolipid bases. J. Lipid Res. 8:391–395.
4. Klenk, H.-D., L. A. Caliguiri, and P. W. Choppin. 1970. The proteins of the parainfluenza virus SV5. II. The carbohydrate content and glycoproteins of the virion. Virology 42:473–481.
5. Klenk, H.-D., and P. W. Choppin. 1971.l Gycolipid content of vesicular stomatitis virus grown in baby hamster kidney cells. J. Virol. 7:416–417.
6. McSharry, J. J., and R. R. Wagner. 1971. Lipid composition of purified vesicular stomatitis viruses. J. Virol. 7:59–70.
7. Reinhold, V. N., F. T. Dunne, J. C. Wriston, M. Schwartz, L. Sarda, and C. H. W. Hirs. 1968. The isolation of porcine ribonuclease, a glycoprotein, from pancreatic juice. J. Biol. Chem. 243:6482–6494.
8. Rothfield, L., and M. Pearlman-Kothencz. 1969. Synthesis and assembly of bacterial membrane components. A lipopolysaccharide-phospholipid-protein complex excreted by living bacteria. J. Mol. Biol. 44:477–492.
9. Wagner, R. R., R. M. Snyder, and S. Yamazaki. 1970. Proteins of vesicular stomatitis virus: kinetics and cellular sites of synthesis. J. Virol. 5:548–558.

Downloaded from http://jvi.asm.org/ on September 28, 2020 by guest