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Comparison of Membrane Protein Glycopeptides of Sindbis Virus and Vesicular Stomatitis Virus

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A comparison has been made of the membrane glycoproteins and glycopeptides from two enveloped viruses, Sindbis virus and vesicular stomatitis virus (VSV). Glycopeptides isolated from Sindbis virus and VSV grown in the same host appear to differ principally in the number of sialic acid residues per glycopeptide; when sialic acid is removed by mild acid treatment, the glycopeptides of the two viral proteins are indistinguishable by exclusion chromatography. Preliminary evidence argues that the carbohydrate moiety covalently bound to different virus-specified membrane proteins may be specified principally by the host.

Carbohydrates have long been recognized as a constituent of enveloped virus particles (7). The analytical work of Frommhagen et al. (2) demonstrated that a polysaccharide of at least four component sugars (galactose, mannose, fucose, and glucosamine) is present in influenza virus particles, accounting for 6 to 7% of the total mass of the particle. Frommhagen et al. (2) called attention to the striking similarities in carbohydrate composition of influenza virus and membrane fragments from uninfected cells. At the time, this similarity was taken as evidence for the incorporation into virus particles of some preformed host polysaccharide.

There is now evidence for another enveloped virus, Sindbis, that all viral carbohydrate is covalently bound to the virus-specified envelope protein (1, 21). Since the Sindbis virus-specified glycoprotein is extensively labeled by monosaccharide precursors added during infection (21), the viral polysaccharide cannot be polymerized cell material synthesized before infection and passively incorporated into viral membrane. The carbohydrate of Sindbis virus appears instead to be an integral part of the virus-specified membrane protein. This result is not unique to arboviruses; most of the carbohydrate of influenza virus is covalently bound to the virus-specified hemagglutinin subunit (9).

The question of current interest is whether the structure of the viral carbohydrate (composition, sequence, and linkages) is specified by the viral genome or the cell. If the cell specified this structure, then a detailed comparison of the glycopeptides from an enveloped virus with glycopeptides from membrane glycoproteins of uninfected host cells should demonstrate similar or identical structures. However, since the glycopeptides of cell membrane glycoproteins have, in general, not been characterized, a detailed comparison of cellular and viral glycopeptides is not immediately possible.

In this study, we have taken a different approach by asking whether the glycopeptides of the membrane glycoproteins of two unrelated enveloped viruses, Sindbis virus and vesicular stomatitis virus (VSV), are similar when grown in the same host; we reasoned that extensive similarities would argue for host determination of the carbohydrate structure. We first determined the identity of the VSV glycoprotein and then compared the glycopeptides of the two viruses with regard to molecular weight and relative amino sugar compositions. The results suggest fundamental similarities in the two viral glycopeptides.

MATERIALS AND METHODS

Cells and viruses. The growth of Sindbis virus (HR strain) in chick embryo fibroblasts has been described (19). VSV, the large plaque variant of the Indiana serotype, was grown in Chinese hamster ovary (CHO) cells or in chick embryo fibroblasts as reported elsewhere (3, 18).

Radioactive labeling and purification of virus. Radioactive amino acids or radioactive glucosamine was added to infected cell cultures immediately after viral attachment to label Sindbis virus and 4 hr after viral attachment to label VSV. Final concentrations of radioactivity were 1.0 μCi/ml of ^{3}H-leucine, 1.0 μCi/ml of ^{3}H-amino acids, 0.1 μCi/ml of ^{14}C-amino acids, 1.0 μCi/ml of ^{3}H-glucosamine, and 3.0 μCi/ml of ^{14}C-glucosamine. Radioactive virus was harvested and purified as usual (18, 20). Because the proteins of

B and T particles of VSV are identical (23), sucrose gradient-purified B particles alone or mixed B and T particles were used in these studies.

Acrylamide gel electrophoresis. Before electrophoresis, purified virus was disrupted by treatment with 1% sodium dodecyl sulfate (SDS) and 1% mercaptoethanol and heated to 90 C for 1 min. The sample was then layered onto a 10% acrylamide gel (linked with 0.3% ethylene diacrylate) which had been prerun in the presence of glutathione, as described previously (20). Electrophoresis was for 4 hr at 8 ma. Gel slices (1 mm) were made by a razor blade device (designed by B. W. Burge and sold by Baruch Instruments, Ossining, N.Y.), dissolved in 0.5 ml of concentrated NH_4OH and, after the addition of a Triton X-100-tolulene-based scintillation fluid (10), counted in a Packard Tri-Carb scintillation counter. All the values have been corrected for background and spillage of ^3H or ^{14}C.

Biogel chromatography. Purified virus was first extensively digested with Pronase (1) and then applied onto a column (45 by 1 cm) packed with Biogel P-10 which had a void volume of 18.5 ml, as indicated by dextran blue 2,000. Sucrose and fetuin glycopeptides were used as additional markers and were detected by the anthrone reaction, and sialic acid by the Warren thiobarbituric acid assay as described by Spiro (16). Samples were eluted from the column with 0.1 M sodium phosphate buffer (*p*H 7.8) at a flow rate of 3 to 4 ml per hr. Fractions of about 0.5 ml were collected automatically, suspended in 10 ml of Triton-tolulene scintillation solution, and counted.

Chromatography of sialic acid and amino sugars. Purified virus preparations were precipitated with ethanol and resuspended in 1 ml of 0.1 N HCl containing 100 μg *N*-acetylneuraminic acid added as carrier. Sialic acid was released by mild hydrolysis (80 C, 1 hr, N_2), and the released sialic acid was separated from glycopeptides by passage through Biogel P-2 (which excludes molecules larger than 1,600 daltons). Glycopeptides in the void volume were hydrolyzed in the presence of carrier amino sugars (4 N HCl, 100 C, 4 hr, N_2), dried under N_2, and chromatographed on paper in a solvent system of pyridine–*n*-butanol–water (6:4:3, v/v; reference 24). Radioactivity retarded by the P-2 column was dried under N_2 and chromatographed on Whatman no. 1 paper strips in a solvent system of butyl acetate–acetic acid–water (3:2:1, v/v; reference 13). Paper strips were counted in a Packard windowless gas-flow strip counter.

Materials. ^{14}C-amino acids (reconstituted protein hydrolysate) at a concentration of ~135 mCi/mmole, D-glucosamine-*U*-^{14}C at 40 mCi/mmole, D-glucosamine-6-^3H at ~0.9 Ci/mmole, and ^3H-leucine at 5 Ci/mmole were purchased from New England Nuclear Corp., Boston, Mass. ^3H-amino acids (reconstituted algal hydrolysate) at ~100 mCi/mmole was obtained from Schwarz BioResearch, Orangeburg, N.Y.; and Biogel was from Bio-Rad Laboratories, Richmond, Calif. Fetuin, purified by the Spiro procedure, was purchased from Gibco, Grand Island, N.Y. *Vibrio cholera* neuraminidase was purchased from Calbiochem, Los Angeles, Calif. Poliovirus protein markers were prepared from infected HeLa cells and was kindly donated by Michael Jacobson. Trypsin (2× crystallized) was obtained from Worthington Biochemical Corp., Freehold, N.J.

RESULTS

VSV glycoprotein. In SDS-polyacrylamide gels, the structural proteins of VSV are separated into four polypeptides labeled 1, 2, 3, and 4 in order of increasing mobility (22). To determine whether any of these polypeptides is a glycoprotein, VSV was grown in CHO cells and labeled with ^3H-glucosamine. Purified ^3H-VSV was mixed with another VSV preparation, also grown in CHO cells, but labeled with ^{14}C-amino acids. Acrylamide gel electrophoresis of this mixture showed that only polypeptide 2 contained incorporated glucosamine (Fig. 1). Upon closer examination of Fig. 1, the distribution of the radioactivity of the two isotopes in peak 2 is not identical, as is more clearly seen by plotting the ratio of ^3H to ^{14}C counts for the three principal fractions which make up peak 2 (fractions 23 to 25). The increasing values for $^3H/^{14}C$ suggest that peak 2 contains two protein species, a larger protein and a smaller glycoprotein. Other labeled preparations of VSV gave similar results. However, electrophoresis of disrupted virus followed by staining with Coomassie blue failed to resolve two components in peak 2.

It should be noted that about 5% of the total ^3H-glucosamine-derived radioactivity in Fig. 1 is found at fractions 65 to 75. Glycolipids migrate at this position in the SDS-acrylamide gel system (Prival and Burge, *unpublished data*) and it is possible that this small percentage of counts corresponds to the glycolipid that Klenk and Choppin (*personal communication*) have demonstrated in VSV. Lipid elements of enveloped viruses are, for the most part, synthesized before infection, and turn over slowly. Therefore, glucosamine label present during infection will label chiefly those carbohydrate-containing elements which must be synthesized de novo—the glycoproteins. We made no attempt in this work to characterize or remove the labeled glycolipid.

To estimate the molecular weight of the VSV glycoprotein, ^{14}C-labeled VSV was co-electrophoresed in an acrylamide gel with ^3H-labeled, poliovirus-specific proteins prepared from infected HeLa cells. The mobility of the poliovirus polypeptides (NCVP 1, NCVP 2, VP 0, VP 1, and VP 3) versus their molecular weights is plotted in Fig. 2. By the methods of Shapiro et al. (12), the four VSV polypeptides have estimated molecular weights of 300,000, 73,000, 49,000, and 25,000. Therefore, the glycoprotein, which is part of polypeptide 2, has a molecular weight of 73,000.

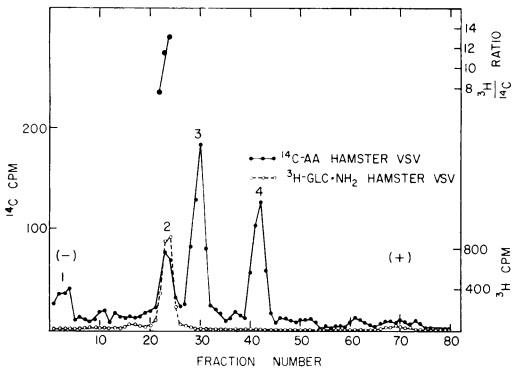

FIG. 1. *Gel electrophoresis of VSV proteins and glycoproteins. VSV grown in CHO cells and labeled with either ^{14}C-amino acids (●) or ^{3}H-glucosamine (○) was disrupted with SDS in a reducing environment and electrophoresed in 10% acrylamide gels. The peak designations (1, 2, 3, 4) follow the convention of Kang and Prevec (8).*

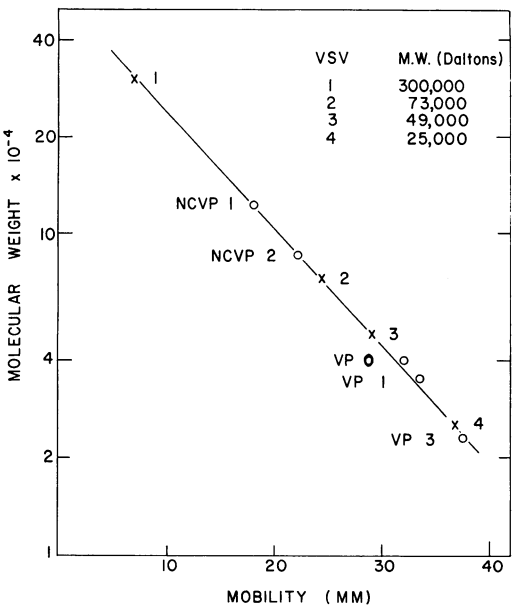

FIG. 2. *Molecular weight of structural proteins of VSV. VSV labeled with ^{14}C-amino acids was prepared for SDS-polyacrylamide gel electrophoresis as previously described (7) except that acetic acid was omitted. The sample was mixed with poliovirus proteins labeled with ^{3}H-leucine, -valine, and -isoleucine and separated on a 7% gel (5 ma, 3.5 hr). Slices (1 mm) of the gel were assayed for radioactivity as previously described (7). The polypeptide pattern is plotted as the mobility in millimeters of the individual polypeptides versus the logarithm of their molecular weights. Symbols: ○, poliovirus polypeptides; ×, VSV polypeptides.*

These determinations of molecular weights for VSV structural proteins are significantly different from those previously reported (23). The poliovirus proteins, used here as standards, had their molecular weights determined with precision (M. F. Jacobson, J. Asso, and D. Baltimore, J. Mol. Biol., *in press*) and compare favorably with other molecular-weight standards which have been extensively calibrated by acrylamide gel electrophoresis (26). Although our estimations appear to be valid, it should be noted that molecular-weight determinations for glycoproteins cannot be considered accurate until there is an understanding of the effects of carbohydrate on electrophoretic mobility of proteins in SDS-containing gels.

Glycopeptides of VSV and Sindbis virus glycoprotein. From the previous experiment on VSV and from studies on Sindbis structural proteins (21), both viruses appear to contain a single species of glycoprotein. Thus, glycopeptides from VSV and Sindbis virus can be compared without first separating and isolating the individual glycoproteins. Tritium and ^{14}C-glucosamine-labeled preparations of the two viruses were simply mixed and digested exhaustively with Pronase; the glycopeptides thus produced were resolved by gel exclusion chromatography.

Sindbis virus glycopeptides have been characterized (1), and therefore Sindbis and VSV glycopeptides, both from virus grown in chick cells, were compared directly by co-chromatography on Biogel P-10. The ^{14}C-glycopeptides of VSV were on the average larger than the ^{3}H-glycopeptides of Sindbis and lacked the small C_C glycopeptide found in Sindbis (Fig. 3a). The VSV glycopeptide peak appeared to co-chromatograph on Biogel P-10 with the fetuin glycopeptide (14) which has a molecular weight of 4,400 daltons. However, the VSV peak is wider than the fetuin peak, indicating size heterogeneity among the VSV glycopeptides.

Previous analysis of Sindbis virus glycopeptides suggested that sialic acid residues attached to glycopeptides cause an increase in molecular weight of glycopeptides sufficient to explain the observed difference in the ratio of elution volume to void volume (V_e/V_0) of glycopeptides from virus grown in chick cells and virus grown in BHK-21 cells (1). To test whether sialic acid was also responsible for the observed difference between glycopeptides of VSV and Sindbis virus, the glycopeptides were subjected to treatment with 0.1 N HCl for 30 min at 80 C, which is sufficient to remove terminally bound sialic acid residues (16). Figure 3b shows the profile of these altered glycopeptides after separa-

tion on Biogel P-10 columns. Both Sindbis and VSV glycopeptides were shifted to lower molecular weights (greater values of V_e/V_0). The small Sindbis glycopeptide, C_C, was still present but no longer well resolved. Removal of sialic acid thus reduced both VSV and Sindbis glycopeptides to the size of the C_B Sindbis glycopeptide, which has a molecular weight of 2,770 daltons (1). In addition, some radioactivity now appeared in the region corresponding to fraction 95, slightly ahead of sucrose, at the elution position of carrier *N*-acetyl neuraminic acid.

The experiment described in Fig. 3b has been repeated, using *V. cholera* neuraminidase to achieve release of sialic acid. The release of counts was slightly less complete with enzyme than with acid, but the fundamental results were not changed.

Molecular weight of glycopeptides and estimate of sialic acid content. Glucosamine and sialic acid were qualitatively identified as glycopeptide constituents both by hydrolysis and chromatography and by release of sialic acid counts from glycopeptide material on neuraminidase treatment. Because of the relatively small percentage of total glucosamine label present as sialic acid, quantitative estimates of this percentage were made from results of Biogel chromatography of glycopeptide preparations subjected to mild acid hydrolysis. The released sialic acid (and carrier sialic acid) appeared on Biogel P-10 columns in a distinctive position, ahead of molecules of the same molecular weight (e.g., sucrose), probably because of the negative charge on the acid. By comparing the area of this peak and that of the glycopeptide (*see* Fig. 3b), a very reproducible estimate of the relative content of labeled sialic acid and glucosamine can be made. Since the uridine diphosphate-glucosamine pool of cells serves as precursor pool for all amino sugars of glycoproteins (including sialic acids; reference 25), radioactivity in glycosamine and sialic acid serves as an approximate indication of their relative chemical amounts. Table 1 indicates that the sialic acid to glucosamine ratio of VSV is several times that of Sindbis virus.

This result is consistent with Fig. 3 in which the shift in Biogel elution position of the VSV glycopeptide on removal of sialic acid seems to be greater than that of the Sindbis glycopeptide. By using the molecular weight for Sindbis glycopeptides previously determined against known markers and a molecular weight of 4,400 daltons for the fetuin glycopeptide (15), an estimate of the molecular weight of the VSV glycopeptide can be made by interpolation, assuming a linear relation between decreasing V_e/V_0 and the log of the molecular weight. These data are compiled in

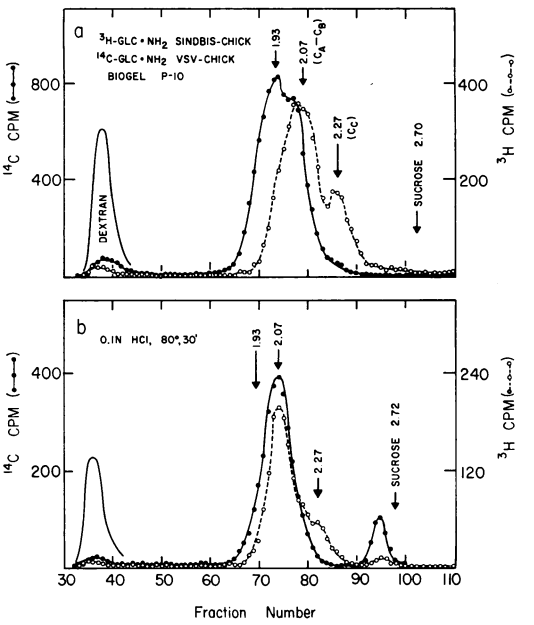

FIG. 3. *Exclusion chromatography of Sindbis and VSV glycopeptides. (a) Sindbis virus grown in chick cells (^{3}H-glucosamine, ○) and VSV grown in chick cells (^{14}C-glucosamine, ●) under identical conditions were mixed and disrupted with 0.5% SDS and exhaustively digested with Pronase. Dextran blue 2,000 and sucrose were used to indicate, respectively, the void volume (V_0) and volume accessible to small molecules. The numbered arrows refer to elution volume/void volume ratios, and C_A, C_B, C_C are designations for Sindbis glycopeptides (1). (b) A portion of the sample studied in part a was hydrolyzed by mild acid treatment (0.1 N HCl, 80 C, 30 min), neutralized, and chromatographed in precisely the same manner as in part a.*

Table 2; it is evident that the average loss in molecular weight of the carbohydrate portion of the VSV glycopeptide after mild acid hydrolysis is on the order of 1,250 daltons. If we assume the loss is solely due to sialic acid residues, the implication would be that the untreated VSV glycopeptide contained approximately four sialic acid residues, each having the molecular weight of 309 daltons.

Unfortunately, this estimate must be considered imprecise for at least two reasons. (i) Anions, such as sialic acid, elute more rapidly from Biogel columns than predicted by molecular weights. If this effect extends to glycopeptides with fixed negative charge, then the loss of charged groups will lead to an overestimate of the shift in molecular weight occurring with this loss. (ii) Loss of charged residues may lead to a rearrangement of glycopeptide structure to a more compact form.

TABLE 1. *Identification of label in virus*[a]

Virus	Host Cells	Per cent of label present as			
		Original label	Glucosamine	Galactosamine	Sialic acid
Sindbis	Chick	^{3}-glucosamine	97	<1	3
VSV	Chick	^{14}-glucosamine	88	<1	12
VSV	Hamster (CHO)	^{3}-glucosamine	85	<1	15

[a] Label was qualitatively identified by hydrolysis and chromatography as described in Materials and Methods. For quantitation it was assumed that label released from glycopeptides by mild acid hydrolysis, which then appeared at V_e/V_o = 2.67, was in the sialic acid form, whereas label remaining in the glycopeptide region was present as glucosamine. Abbreviations: VSV, vesticular stomatitis virus; CHO Chinese hamster ovary.

TABLE 2. *Estimated molecular weights of virus glycopeptides*

Glycopeptide	V_e/V_o	Molecular weight of glycopeptide	Estimated molecular weight of carbohydrate
		daltons	*daltons*
Fetuin	1.91	4,400	3,960
VSV	1.93	4,170	3,750
Sindbis C_A — C_B	2.07	2,770	2,490
Sindbis C_C	2.27	1,840	1,660
VSV and Sindbis after mild acid hydrolysis	2.07	2,770	2,490

[a] All data on the ratio of elution volume to void volume refers to determinations made with Biogel P-10. The molecular weights of the fetuin glycopeptide and Sindbis glycopeptides, C_A, C_B, and C_C, are known from previous work (1, 19). Estimation of the vesticular stomatitis virus (VSV) glycopeptide molecular weight was made by interpolation between known glycopeptides, assuming a negative linear relation between V_e/V_o and log of molecular weight. For the final column, it was assumed that virus glycopeptides were 90% carbohydrate by weight.

This would also tend to produce an overestimate of the number of sialic acid residues removed. Because of these uncertainties, we can only estimate that each VSV glycopeptide has released between two and four residues of sialic acid during mild acid hydrolysis.

Comparison of glycopeptides from VSV grown in chick and hamster cells. In a previous study on glycopeptides of Sindbis virus, it was observed that Sindbis virus grown in hamster cells (BHK) has a glycopeptide population which is larger, on average, than glycopeptides from virus grown in chick cells (1). The difference is accounted for in part by a greater amount of sialic acid in the hamster-grown Sindbis. This same phenomenon is seen in Fig. 4, in which glycopeptides from VSV grown in hamster (CHO) and chick cells are compared. The difference in shape of the ^{3}H- and ^{14}C-glucosamine profiles is not striking, and

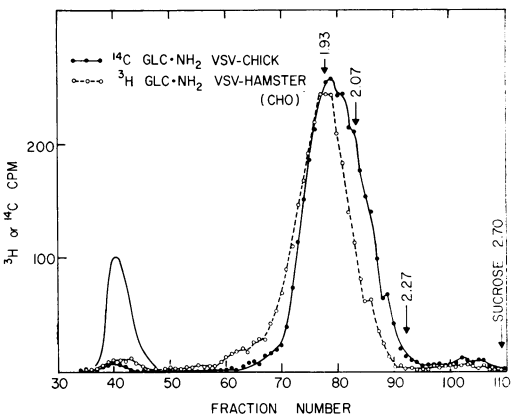

FIG. 4. *Exclusion chromatography of glycopeptides from VSV grown in two different host cells. VSV grown in chick cells (^{14}C-glucosamine, ●) and VSV grown in CHO cells (^{3}H-glucosamine, ○) was mixed, disrupted, digested, and chromatographed on Biogel P-10.*

would no doubt be missed if the two pronase digests were chromatographed independently. Since the two preparations were mixed before digestion, the observed differences in distribution cannot be due to differential digestion, but must be accounted for in terms of differences in carbohydrate composition or structure. After mild acid hydrolysis, both ^{3}H- and ^{14}C-glycopeptide peaks were shifted to a V_e/V_o of 2.07 and were indistinguishable, suggesting that the small difference seen in Fig. 4 was due to slightly different amounts of sialic acid in the two preparations. In agreement with the result, Table 1 indicates that the VSV from hamster cells (CHO) has a slightly higher content of labeled sialic acid than VSV grown in chick cells.

With both VSV preparations, the small glycopeptide C_C seen in glycopeptide preparations from Sindbis grown either in chick or hamster cells (e.g., Fig. 3a) was absent.

DISCUSSION

Universality of glycoproteins in virus membranes. It is becoming apparent that carbohydrate

is a universal constituent of the lipoprotein membrane of all classes of enveloped animal viruses. In several instances it has been demonstrated, by using acrylamide gel techniques, that the majority of the virus carbohydrate is covalently bound to a membrane protein. The list of glycoprotein-containing viruses includes arboviruses of groups A and B (19, 21), influenza virus (8), herpesvirus (13), rhabdovirus, or VSV-like viruses (this paper), and poxviruses (J. A. Holowczak, Bacteriol. Proc., p. 189, 1970). It is expected that avian leukosis viruses will soon be added to the list.

In the case of animal viruses without envelopes, carbohydrate has not yet been demonstrated. It is also of interest that the nucleocapsids of both Sindbis and VSV, structures which are analogous to the infectious nucleocapsids of nonenveloped viruses, are not labeled by glucosamine. In the case of Sindbis virus, it is clear that the glycoprotein is in the viral envelope (22). However, with VSV, the association of glycoprotein with only membrane material has not been clearly established.

The presence of glycoprotein in all viral envelopes thus far studied suggests some critical but, as yet, undetermined biological function for carbohydrate. Recently, Kang and Prevec (7) showed that a soluble glycoprotein produced during VSV infections and similar to virus-associated glycoprotein, contains serum blocking power in a neutralization test with VSV. Whether the carbohydrate or the protein moiety is responsible for the interaction with antiserum is not known.

Degree of similarity of the glycopeptides of VSV and Sindbis virus. The description of the carbohydrate structures of Sindbis and VSV in this paper is incomplete, and is preliminary to a precise determination of carbohydrate composition by gas-liquid chromatography and identification of some of the oligosaccharides of the virus glycopeptides (*unpublished data*). Nonetheless, a summary of our knowledge of these carbohydrate structures will permit certain generalizations. Both virus glycopeptides include glucosamine and sialic acid but not galactosamine, whether the virus is grown in avian or mammalian cells. In addition, the Sindbis glycopeptide includes mannose, galactose, and fucose. These sugars may be present but have not yet been tested for in VSV glycopeptides.

Although glycopeptides of membrane proteins of chicken and hamster cells cover a spectrum of molecular weights from 1,000 daltons to more than 6,000 daltons (1), the glycopeptides of Sindbis and VSV grown in these cells form a very limited subset in terms of size; when sialic acid is removed from virus glycopeptides by hydrolysis or neuraminidase treatment, a single major glycopeptide peak is observed, regardless of the strain of virus or host in which it was grown.

Glycopeptides can be distinguished by more subtle criteria; for instance, either Sindbis or VSV grown in hamster cells is found to have larger glycopeptides than the same virus grown in chick cells, most probably because of an increase in the number of terminal sialic acid residues. When Sindbis and VSV are both grown in chick cells, VSV is found to have larger glycopeptides than Sindbis, again most probably because of an increase in the number of terminal sialic acid residues per glycopeptide. Finally, Sindbis virus grown in either chick or hamster cells has minor amounts of a small glycopeptide (C_C; 1,740 daltons) containing only mannose and glucosamine; this glycopeptide is not seen in VSV preparations from either cell.

These observations can be unified by assuming that the glycopeptides of both viruses, from either host cell, have the same basic structure in terms of the identities and location of individual sugar residues. However, this structure is assembled not on a template but by a family of sugar transferases which act in consort to produce the complete carbohydrate structure (17). Thus, depending on the disposition of these enzymes in the host cell and the peculiarities of synthesis and maturation of the membrane glycoprotein of each virus, structures identical in terms of sequence and linkage of sugars may reach a degree of completion which is characteristic of both virus and cell. A phenomenon similar to the events proposed above has been termed microheterogeneity by those who study the glycopeptides of serum glycoproteins (11). In the case of Sindbis virus, this phenomenon can explain the presence of the small C_C glycopeptide (Fig. 3) which is presumed to be the glucosamine-mannose core of the larger, more complete glycopeptides, C_A and C_B (1).

Host modification. In the above discussion we made the implicit assumption that the enzymes which assemble the virus glycoprotein structure (nucleotide sugar transferases) are specified by the host genome. This is assumed for two reasons. (i) A large number, at lease six transferases, would be required for formation of a carbohydrate structure of the complexity of the Sindbis glycopeptide, and it seems unreasonable that a virus genome of 4×10^6 to 5×10^6 daltons would specify this number of transferases. (ii) The details of virus glycopeptide structure presently available suggest that virus glycopeptides are very similar to the prototypical glycopeptide structure found in serum glycoproteins of all vertebrates. This structure consists of a glucosamine-mannose core linked covalently through glucosamine by a *N*-glycosidic bond to the amide

nitrogen of asparagine in the polypeptide chain (17). To the core are attached three or four chains of the sequence: glucosamine, galactose, with fucose or sialic acid as the terminal nonreducing sugars.

Although it is by no means proven, it seems most reasonable to assume that this structure is determined by host enzymes and that the glycopeptide represents a covalent host modification of a virus-specified protein. It has been demonstrated with serum glycoproteins that the glycopeptide is attached at a specific amino acid sequence, (CHO)-Asn-*x*-Thr, where *x* is any amino acid (5). To proceed with the assumption of host modification, one must infer that the virus-specified protein has evolved such an amino acid sequence to serve as substrate for the first of the host-glycosylating enzymes, or, alternatively, that the virus specifies one sugar transferase to catalyze the formation of the initial sugar-amino acid bond, and the host enzymes then complete the structure.

If the virus carbohydrate is in fact a host modification, many reports of the presence of host antigen in purified virus preparations must necessarily be reinterpreted. Some of these reports no doubt stem from incomplete purification; it is possible, however, that virus membrane protein may behave as a hybrid antigen, part host-specified and part virus-specified. The immunological consequences of such a situation are by no means apparent; such a hybrid antigen could conceivably stimulate formation of antibodies directed against carbohydrate-containing structures of uninfected host cells.

Despite absence of any known functions for the carbohydrate moiety of viral membrane proteins, phenomena such as the exit of enveloped viruses from cells, resistance of virions to environmental hazards, and attachment and penetration of viruses into new hosts may all be expected to have some basis in the physical and chemical structure of the viral surface.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Burge, B. W., and J. H. Strauss, Jr. 1970. Glycopeptides of the membrane glycoprotein of Sindbis virus. J. Mol. Biol. 47:499–466.
2. Frommhagen, L. H., C. A. Knight, and N. K. Freeman. 1959. The ribonucleic acid, lipid and polysaccharide constituents of influenza virus preparations. Virology 8:176–197.
3. Huang, A. S., J. W. Greenawalt, and R. R. Wagner. 1966. Defective T particles of vesicular stomatitis virus. I. Preparation, morphology, and some biologic properties. Virology 30:161–172.
4. Huang, A. S., and D. Baltimore. 1970. Initiation of polyribosome formation in poliovirus-infected HeLa cells. J. Mol. Biol. 47:275–291.
5. Jackson, R. L., and C. H. W. Hirs. 1970. The primary structure of porcine pancreatic ribonuclease. I. The distribution and sites of carbohydrate attachment. J. Biol. Chem. 245: 624–636.
6. Jacobson, M. F., and D. Baltimore. 1968. Morphogenesis of poliovirus. I. Association of the viral RNA with coat protein. J. Mol. Biol. 33:369–378.
7. Kang, C. Y., and L. Prevec. 1970. Proteins of the vesicular stomatitis virus. II. Immunological comparisons of viral antigens. J. Virol. 6:20–27.
8. Knight, C. A. 1947. The nucleic acids and carbohydrates of influenza virus. J. Exp. Med. 85:99–116.
9. Laver, W. G., and R. G. Webster. 1966. The structure of influenza virus. Virology 30:104–115.
10. Patterson, M. S., and R. C. Greene. 1965. Emulsion counting techniques for aqueous ^{3}H and ^{14}C-labeled samples. Anal. Chem. 37:854–857.
11. Reinhold, V. N., F. T. Dunne, J. C. Wriston, M. Schwarz, L. Sarda, and C. H. W. Hirs. 1968. The isolation of porcine ribonuclease, a glycoprotein, from pancreatic juice. J. Biol. Chem. 243:6482–6494.
12. Shapiro, A. L., E. Vinuela, and J. V. Maizel, Jr. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28:815–820.
13. Shen, L., and V. Ginsberg. 1967. Sugar analysis of cells in culture. Arch. Biochem. Biophys. 122:474–480.
14. Spear, P. G., J. M. Keller, and B. Roizman. 1970. Proteins specified by herpes simplex virus. J. Virol. 5:123–131.
15. Spiro, R. G. 1965. Studies on fetuin, a glycoprotein of fetal serum. J. Biol. Chem. 237:382–388.
16. Spiro, R. G. 1966. Analysis of sugars found in glycoproteins, p. 3–60. *In* E. F. Neufeld and V. Ginsburg (ed.), Methods in enzymology, vol. 8. Academic Press Inc., N.Y.
17. Spiro, R. G. 1969. Glycoproteins: their biochemistry, biology and role in human disease. N. Eng. J. Med. 281:991–1000.
18. Stampfer, M., D. Baltimore, and A. S. Huang. 1969. Ribonucleic acid synthesis of vesicular stomatitis virus. I. Species of ribonucleic acid found in Chinese hamster ovary cells infected with plaque-forming defective particles. J. Virol. 4:154–161.
19. Stollar, V. 1969. Studies on the nature of Dengue virus. IV. The structural proteins of type 2 Dengue virus. Virology 39:426–438.
20. Strauss, J. H., Jr., B. W. Burge, and J. E. Darnell. 1969. Sindbis virus infection of chick and hamster cells: synthesis of virus specific proteins. Virology 37:367–376.
21. Strauss, J. H., Jr., B. W. Burge, and J. E. Darnell. 1970. Carbohydrate content of the membrane protein of Sindbis virus. J. Mol. Biol. 47:437–448.
22. Strauss, J. E., B. W. Burge, E. R. Pfefferkorn, and J. E. Darnell. 1968. Identification of the membrane protein and core protein of Sindbis virus. Proc. Nat. Acad. Sci. U.S.A. 59:533–537.
23. Wagner, R. R., T. A. Schnaitman, and R. M. Snyder. Structural proteins of vesicular stomatitis viruses. J. Virol. 3:395–403.
24. Wagner, R. R., T. C. Schnaitman, R. M. Snyder, and C. A. Schnaitman. 1969. Protein composition of the structural components of vesicular stomatitis virus. J. Virol. 3:611–618.
25. Warren, L. 1966. The biosynthesis and metabolism of amino sugars and amino sugar-containing compounds, p. 570–590. *In* A. Gottschalk (ed.), Glycoproteins. Elsevier Publishing Co., New York.
26. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406–4412.