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Purification and Composition of the Proteins from Sindbis Virus Grown in Chick and BHK Cells

DAVID J. BURKE* AND KENNETH KEEGSTRA
Department of Microbiology, State University of New York, Stony Brook, New York 11794

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Procedures are described for the purification of the Sindbis virus structural proteins. The amino acid and carbohydrate compositions of the purified proteins are presented for virus grown in BHK-21/13 and chicken embryo cells. Glycoprotein E1 from virus grown in BHK cells is deficient in a mannose-rich glycopeptide found on that glycoprotein when virus is grown in chicken embryo cells. The complex galactose-containing glycopeptides appear similar for virus grown in both hosts. However, when virus is grown in BHK cells, both glycoproteins are enriched in those glycopeptides containing more sialic acid. Since the two viral glycoproteins are difficult to separate cleanly during purification, it is suggested that there may be strong, but noncovalent, interactions between glycoproteins E1 and E2. It is also suggested that there may be an interaction between glycoprotein E2 and a component of the nucleocapsid.

Sindbis is an alphavirus or group A togavirus. The virion is comprised of a nucleocapsid surrounded by a lipid envelope from which glycoprotein spikes protrude (5, 13). The nucleocapsid contains the single-stranded 42-49*S* virion RNA and a single nucleocapsid core protein with a molecular weight of approximately 30,000 (13, 30). The envelope is similar in lipid composition to the plasma membrane of the vertebrate host cell in which the virus was produced (7, 15). The two virus-specific envelope glycoproteins, E1 and E2, have molecular weights of 53,000 and 45,000, respectively (25). The lipid envelope and the glycoproteins are acquired by the nucleocapsid as it buds through the host plasma membrane to form the mature virus particle (1, 2).

During infections of vertebrate host cells, the Sindbis glycoproteins are synthesized, glycosylated, and transported to the cell surface in a manner that closely resembles the processing of host cell glycoproteins (29). Since the virus has only a very limited amount of genetic information, it has been argued that the enzymes that glycosylate the viral glycoproteins must be principally, if not entirely, host specific (16). Therefore, Sindbis and the other togaviruses provide unique probes for studying the processes by which different host cells glycosylate the same viral polypeptides (18, 28).

When Sindbis virus is grown in secondary chicken embryo cells, glycoproteins E1 and E2 both contain two different types of oligosaccharide structures. One of these, called an A-type oligosaccharide, contains glucosamine, mannose, galactose, and sometimes sialic acid and fucose. The other, a B-type oligosaccharide, contains only glucosamine and mannose (28). Each glycoprotein contains three different A-type oligosaccharide species, which differ principally in the degree to which they are sialylated (18). The B-type oligosaccharides are also somewhat heterogeneous and are rich in mannose (28).

Etchison and Holland (9, 10) reported that the glycoprotein of vesicular stomatitis virus has a similar carbohydrate composition when the virus is grown in four different mammalian cell types. However, small host-specific differences were seen. Similarly, Sindbis virus grown in BHK-21 cells has a somewhat different carbohydrate composition than virus grown in chicken embryo cells (18, 34). Specifically, the virus grown in BHK cells contains less mannose, but more galactose and sialic acid, than virus from chick cells (18). In this report, it is shown that the decreased mannose content of BHK-grown virions results from a reduction in the amount of B-type glycopeptides on glycoprotein E1. This is shown both by the carbohydrate compositions of the purified glycoproteins and by analysis of the glycopeptides isolated from the purified glycoproteins after treatment with proteases. Except for the deficiency of mannose-rich oligosaccharide structures on glycoprotein E1, the glycopeptides of virus grown in the two hosts are very similar.

A scheme for the large-scale purification of viral proteins is presented. This procedure involves disruption of the virion with Triton X-

100 at high ionic strength, removal of nucleocapsids by centrifugation, and purification of the glycoproteins by ion-exchange chromatography. An alternate procedure for separating glycoprotein E1 from glycoprotein E2 and core protein by its solubility in detergent at low ionic strength is also described. Aspects of these purification schemes are discussed with regard to their implications about virion structure. Amino acid compositions of the purified virion proteins are presented. These proteins have compositions very similar to those of analogous structural proteins from Semliki forest virus (12, 31).

MATERIALS AND METHODS

Growth and purification of Sindbis virus. Secondary chicken embryo cells were prepared as described previously (28) and were grown at 37°C in Eagle medium (Grand Island Biological Co. [GIBCO], F-11) supplemented with 2% tryptose phosphate broth, 1% calf serum, and 1% heat-inactivated chicken serum (GIBCO). BHK-21/13 cells were grown at 37°C in Eagle medium supplemented with 5% fetal calf serum (GIBCO). Nonradioactive Sindbis was produced by two steps of growth in monolayers of cells in glass roller bottles (28). Radioactive virus was produced by a single step of growth in monolayers of cells in 100-mm Falcon tissue culture plates. In the latter case, cells were infected with a multiplicity of 10 to 50 PFU/cell. After 1 h, they were incubated in medium containing 10 μCi of [6-^{3}H]glucosamine per ml (New England Nuclear Corp., 10 Ci/mmol), 2.5 μCi of [1-^{14}C]glucosamine per ml (New England Nuclear Corp., 56.5 mCi/mmol), 5.0 μCi of [1-^{14}C]galactose per ml (New England Nuclear Corp., 53 mCi/mmol), or 10 μCi of [2-^{3}H]mannose per ml (New England Nuclear Corp., 2.0 Ci/mmol).

Virus labeled with radioactive amino acids was prepared in a similar manner, using medium containing 10 μCi of a ^{14}C-labeled amino acid mixture (New England Nuclear Corp., NEC-445) and 1.0 μg of actinomycin D per ml. At 12 h after infection, the labeled virus was harvested and purified as described previously (28).

Purification of radiolabeled Sindbis virus glycoproteins by SDS-polyacrylamide gel electrophoresis. Radioactive amounts of Sindbis glycoproteins E1 and E2 were purified by slicing and extracting cylindrical sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis gels, which were prepared and run as described previously (28).

Analysis of protein purity on discontinuous slab gels. Analytical SDS-polyacrylamide slab gels were prepared using the same stacking and resolving gels as were used for the cylindrical gels described above. The gels were 0.2-cm thick, and the resolving and stacking gels were 8.0 and 1.5 cm in length, respectively. After the stacking gels had hardened, the gels were overlaid with electrode buffer. This electrode buffer was also the same as that described previously (28). Samples were reduced by dissolving in 0.5 M Tris, pH 6.8, containing 1% (wt/vol) sodium lauryl sulfate, 10% (wt/vol) glycerol, and 1% 2-mercaptoethanol, followed by heating to 100°C for 2 min. The dissolved samples were carefully layered on top of the stacking gels and beneath the electrode buffer. The electrophoresis was started at a potential of 150 V and was kept at that voltage until all samples entered the stacking gel. The voltage was then reduced to 125 V and run to completion. Typical electrophoresis time was 2 h. Gels were stained overnight with 0.25% Coomassie brilliant blue R (Sigma) in water-methanol-acetic acid (5:5:1) and destained with water-methanol-acetic acid (17:1:2). Gels to be autoradiographed were dried onto filter paper and then exposed to Kodak SB-54 X-ray film.

Amino acid analysis. Samples containing 100 μg of protein were hydrolyzed for 22 h at 100°C in 0.5 ml of 6 N hydrochloric acid. After hydrolysis, samples were diluted with 3 volumes of distilled water, frozen at −80°C, and lyophilized. These hydrolysates were analyzed by using a Beckman amino acid analyzer equipped with an automated integrator. Peaks were identified, and weights of amino acids were quantitated by comparison with authentic amino acid standards.

Analysis of monosaccharide and sialic acid compositions. Samples to be analyzed for monosaccharide composition were hydrolyzed with 2 N trifluoroacetic acid (Mallinkrodt) for 2 h at 121°C, and the alditol acetate derivatives of the resulting sugars were prepared as described previously (18). These alditol acetates were separated by use of a Hewlett-Packard 5710A gas chromatograph using glass columns (6 feet by 0.125 inch [ca. 183 by 0.32 cm]) packed with 1% OV-275 (Applied Science) on Gas Chrom Q (Applied Science). The columns were eluted at a nitrogen carrier gas-flow rate of 60 ml/min. After injecting the sample at 140°C, the columns were maintained at this temperature for 4 min, and then the oven temperature was raised at a linear rate of 1°C/min to a final temperature of 220°C, at which temperature the oven remained for 8 min. Peak areas were integrated by use of a Hewlett-Packard 3370-B integrator, and masses of the component sugars were quantitated by comparison with a myo-inositol internal standard. Sialic acids were determined either by the procedure of Warren (38) or by the procedure of Reinhold (21).

Analysis of viral glycopeptides by chromatography on Bio-Gel P-6. Radiolabeled viral glycoproteins were digested with protease from *Streptomyces griseus* (Sigma) at 60°C for 24 h (28, 32). The resultant glycopeptides were chromatographed on a Bio-Gel P-6 column (200 to 400 mesh, Bio-Rad) measuring 1.0 by 120 cm, using 0.1 M Tris-hydrochloride, pH 8.0, as the eluting buffer. Fractions (0.5 ml) were collected directly into shell vials, combined with 3.0 ml of Triton-toluene (1:1, vol/vol) scintillation cocktail [6.0 g of 2,5-diphenyloxazole and 0.25 g of 1,4-bis-(5-phenyloxazolyl)benzene per liter], and counted in a Nuclear-Chicago scintillation counter.

RESULTS

Solubilization of the Sindbis glycoproteins using Triton X-100. After purification, unla-

beled Sindbis virus and [^{3}H]glucosamine-labeled virus were combined so that the resulting mixture contained 4,000 cpm/mg of glycoprotein. This preparation was precipitated by addition of 10 volumes of cold water followed by centrifugation. The pellet was then resuspended at a protein concentration of 1.0 mg/ml by sonication in a solution of 0.2 M Tris buffer, pH 8.0, containing 0.5% Triton X-100. More than 80% of the two glycoproteins were solubilized (Fig. 1, lane 4), whereas all of the core protein and small amounts of glycoproteins E1 and E2 remain in the pellet after centrifugation at 160,000 × *g* for 1 h (Fig. 1, lane 5). A second resuspension of the 160,000 × *g* pellet in this same solution resulted in solubilization of all the remaining glycoproteins, leaving a pellet containing only core protein and probably the virion RNA. Core protein purity is shown in Fig. 2. Suspension of Sindbis in 0.2 M phosphate buffers at pH 7.0 and 6.0 resulted in a less complete solubilization of the envelope glycoproteins.

DEAE-Sephadex column chromatography of detergent-solubilized glycoproteins. Purified Sindbis virus preparations containing approximately 50 mg of protein were pooled, and glycoproteins E1 and E2 were solubilized in Triton X-100 at a protein concentration of 1 mg/ml, with core protein removed as described above. If this solution is applied to a DEAE-Sephadex A-50 column, neither of the two glycoproteins is retained on the column. However, if the buffer concentration was first lowered to 0.04 M by addition of aqueous 0.5% Triton X-100, two peaks could be resolved by chromatography on DEAE-Sephadex A-50. Figure 3 shows the chromatography of glycoproteins E1 and E2 of virus grown in chicken embryo cells on a 1.5- by 40-cm DEAE-Sephadex column that had

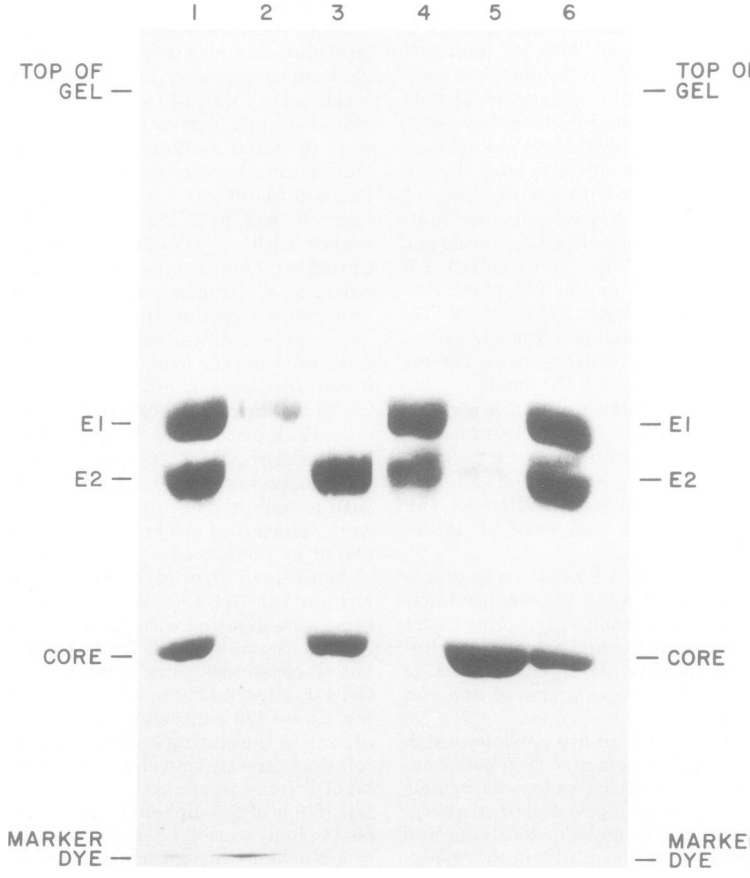

FIG. 1. *Solubilization of Sindbis glycoproteins in 0.5% Triton X-100 as a function of pH and ionic strength. This figure shows an autoradiogram of* ^{14}*C-amino acid-labeled Sindbis proteins analyzed by SDS-polyacrylamide gel electrophoresis. Lanes 1 and 6 are intact virus proteins run as markers. Lanes 2 and 3 are the supernatant and pellet, respectively, of virus solubilized in 0.01 M phosphate buffer at pH 6.0. Lanes 4 and 5 are the supernatant and pellet of virus solubilized in 0.20 M Tris-hydrochloride buffer at pH 8.0.*

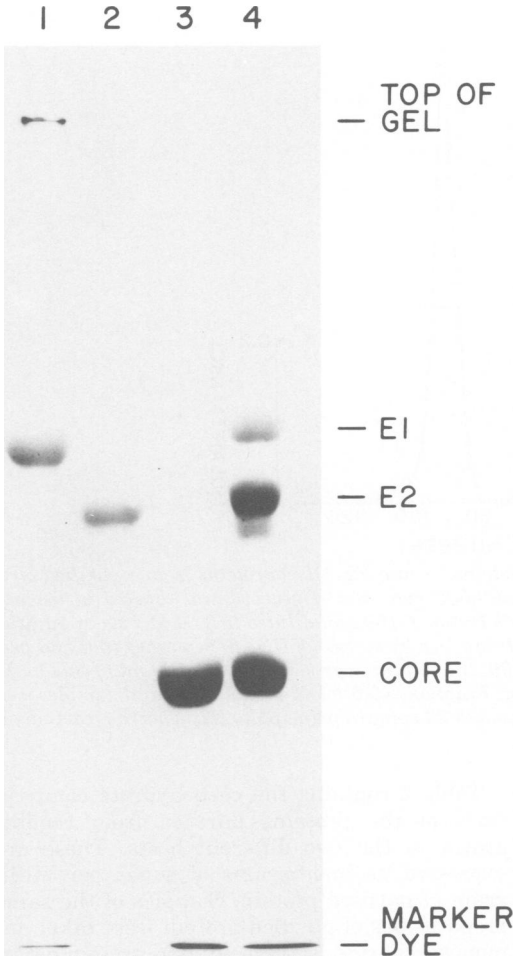

FIG. 2. *Purified Sindbis proteins. This is Coomassie brilliant blue-stained, analytical SDS-polyacrylamide gel electrophoresis of the Sindbis virion proteins purified by solubilization in Triton X-100 and DEAE-Sephadex A-50 column chromatography. Lane 1 is purified glycoprotein E1; lane 2 is purified glycoprotein E2; lane 3 is purified core protein; lane 4 is whole virus.*

been equilibrated with 0.04 M Tris, pH 8.0, containing 0.5% Trition X-100. The first peak eluted at the column void and contained most of the original glycoprotein E2 and about 10% of the glycoprotein E1. A second peak eluted with the addition of buffer plus 0.2 M sodium chloride in 0.5% Trition X-100. This peak contained most of the glycoprotein E1, with a small amount of glycoprotein E2. In early experiments, a linear gradient was used for elution of the column; however, a stepwise gradient was subsequently used, with similar results.

The fractions enriched in glycoprotein E2 (fractions 5–34, Fig. 3) were pooled and run through a second column of DEAE-Sephadex A-50 to remove the contaminating glycoprotein E1. The pooled glycoprotein E1 fractions (fractions 81–96, Fig. 3) were diluted with 5 volumes of aqueous 0.5% Triton X-100 and applied to a second DEAE-Sephadex A-50 column equilibrated with 0.04 M Tris, to remove the small amount of contaminating glycoprotein E2. The pure E1 was then eluted by washing this column with 0.04 M buffer plus 0.2 M sodium chloride as before. The purity of glycoproteins E1 and E2 after this purification is shown in Fig. 2.

Alternate procedure for the purification of glycoprotein E1. When Sindbis is suspended in 0.5% Triton X-100 in 0.01 M phosphate buffer, pH 6.0, rather than 0.2 M Tris, pH 8.0, greater than 90% of glycoprotein E1 is solubilized. However, all of glycoprotein E2 plus all of the core protein are recovered in the 160,000 × *g* pellet, along with a small amount of glycoprotein E1. A second resuspension of this pellet with the same buffer is sufficient to solubilize the remainder of glycoprotein E1, yielding a pellet containing only glycoprotein E2 plus core protein. Figure 1 (lanes 2 and 3) shows glycoprotein E1 and the mixture of glycoprotein E2 plus core protein prepared in this manner. This procedure is of great utility if one wishes only to purify the glycopeptides from each glycoprotein, since it is much simpler than the column procedure described above. Since the core protein is not glycosylated, the glycopeptides from glycoprotein E2 can be obtained by simply digesting with protease the mixture of core protein and glycoprotein E2.

Attempts to selectively solubilize glycoprotein E2 from the pellet containing the mixture of glycoprotein E2 and core protein have not been successful. The use of 0.2 M Tris at pH 8.0, which will solubilize both glycoproteins E1 and E2 from the intact virion, leaves both the core protein and glycoprotein E2 insoluble. The use of buffer plus 1 M NaCl solubilizes a large proportion of both glycoprotein E2 and core protein. The above results could be explained either by a specific interaction between glycoprotein E2 and the viral nucleocapsid at low ionic strength or by an aggregation of E2 at low ionic strength, leading to its co-precipitation with nucleocapsids. To determine whether nucleocapsids were necessary for pelleting E2 at low ionic strength, the following experiment was performed. Pure glycoprotein E2 (30 μg of E2 per ml) in 0.04 M Tris, pH 8.0, containing 0.5% Triton X-100 was prepared by DEAE-Sephadex chromatography as described above. A pellet containing 400 μg of core protein was suspended by sonication in 5 ml of this E2 prep-

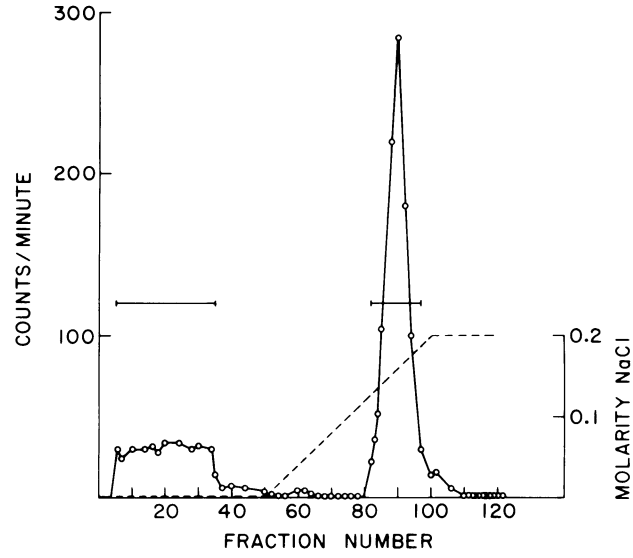

FIG. 3. *DEAE-Sephadex chromatography of glycoproteins E1 and E2. Glycoproteins from a purified virus preparation containing 50 mg of virus protein and 200,000 cpm of [^{3}H]glucosamine-labeled virus were solubilized in 50 ml of 0.2 M Tris, pH 8, containing 0.5% Triton X-100 and diluted to 0.04 M Tris by addition of 0.5% Triton X-100. This solution was applied to a column (1.5 by 40 cm) of DEAE-Sephadex that had been equilibrated against 0.04 M Tris plus 0.5% Triton X-100; the column was washed with 100 ml of this buffer plus detergent, and then a linear salt gradient was run. Fractions (5.0 ml) were collected and samples were assayed by liquid scintillation counting. Fractions 5 through 34 contain principally E2, whereas fractions 81 through 96 contain principally E1.*

aration and centrifuged at 160,000 × *g* for 1 h as before. Under these conditions, approximately 20% of the E2 pelleted with the nucleocapsids. In a control lacking nucleocapsids, no pellet was formed and all of the E2 remained soluble in the supernatant. This experiment demonstrates that some component of the nucleocapsid is essential for pelleting E2 at low ionic strength. However, since the core protein has not been purified away from the virion RNA, it is possible that either the core protein or the RNA is the essential component. In addition, the lipid contents of the E2 and nucleocapsid preparations are not known.

Chemical characterization of the Sindbis virion proteins. The amino acid compositions of the Sindbis proteins purified from virus grown in BHK and chicken embryo cells are shown in Table 1. Determinations were made as described in Materials and Methods. These amino acid compositions do not include data for cysteine or tryptophan. The data are expressed as moles percent of total amino acids. Difficulty was encountered in quantitating the methionine peaks in samples of E1 and E2 from virus grown in BHK cells. These peaks were quite small and were only partially resolved from a peak associated with a buffer change in those analyses. Each value in Table 1 represents an average of two determinations.

Table 2 contains the carbohydrate compositions of the proteins purified from Sindbis grown in the two different hosts. These are expressed as micrograms of sugar per milligram of purified protein. Samples of the same preparations of purified protein were taken for monosaccharide, sialic acid, and protein determinations. Monosaccharide and sialic acid values were obtained as described in Materials and Methods. Monosaccharide and sialic acid values represent averages of a minimum of two different determinations performed in duplicate on independently purified preparations. Protein content was analyzed by the method of Lowry et al. (19). Each preparation of E2 from a given host contains the same proportions of glucosamine, mannose, and galactose, but the absolute values of these sugars in micrograms of sugar per milligram of protein varied somewhat from experiment to experiment. Glucose values varied considerably for both glycoproteins from both hosts. Some samples contained almost no glucose, whereas others contained as much as 11 μg/mg of protein. This sugar may be present primarily as a contaminant resulting from the DEAE-Sephadex column chromatography step, since it is not present in the glycopeptides purified after protease treatment of the glycoproteins (Burke and Keegstra, unpublished data).

TABLE 1. *Moles percent of amino acids in Sindbis virion proteins*

Amino acid	Glycoprotein E1		Glycoprotein E2		Core protein	
	BHK	Chick	BHK	Chick	BHK	Chick
Lysine	6.60	5.92	5.97	6.47	8.59	8.10
Histidine	2.80	3.29	3.24	4.71	2.58	2.55
Arginine	3.26	2.59	4.39	4.95	8.17	6.94
Aspartate	9.05	8.30	10.42	8.92	7.19	8.33
Threonine	7.76	7.90	9.35	8.97	6.64	6.11
Serine	9.23	9.58	8.19	7.64	5.12	6.22
Glutamate	9.10	7.15	8.00	6.65	10.33	8.99
Proline	6.93	5.28	7.60	5.87	9.73	8.31
Glycine	7.59	7.00	6.87	6.56	10.41	9.24
Alanine	10.24	10.88	8.79	8.06	8.59	7.99
Valine	7.26	6.81	7.21	6.52	5.54	5.73
Methionine	0.66[a]	4.18	0.31[a]	3.94	2.07	1.81
Isoleucine	5.51	4.96	5.60	5.77	3.34	4.01
Leucine	6.83	6.26	6.79	7.65	6.09	7.53
Tyrosine	3.21	3.50	4.34	4.54	1.86	2.45
Phenylalanine	3.94	4.45	2.93	3.03	3.64	3.38

[a] Probably low estimates; see text.

TABLE 2. *Carbohydrate compositions of the Sindbis glycoproteins isolated from BHK-21 or chicken embryo cells*

Sugar	μg of sugar/mg of protein			
	Glycoprotein E1		Glycoprotein E2	
	Chick cells	BHK-21	Chick cells	BHK-21
Glucosamine	23.5	24.2	21.4	18.9
Mannose	24.2	18.9	24.9	25.9
Galactose	8.3	12.2	5.7	8.6
Fucose	2.5	2.8	<1.0	1.1
Sialic acid	4.7	5.3	3.5	6.5
Glucose	5.8	5.6	7.1	6.5
Total (minus glucose)	63.2	63.4	55.5	61.0

Analysis of the glycopeptides of the purified envelope proteins from Sindbis grown in BHK cells. The mannose content of glycoprotein E1 from virus grown in BHK cells is much reduced relative to E1 from virus grown in chicken embryo cells (see Table 2). This result suggests that glycoprotein E1 from virus grown in BHK cells might be deficient in the mannose-rich glycopeptide S4, present when virus is grown in chicken embryo cells (28). To test this hypothesis, Sindbis was grown in BHK cells and labeled with [^{14}C]glucosamine, whereas virus grown in chicken embryo cells was labeled with [^{3}H]glucosamine. Glycoproteins E1 and E2 were purified from each virus preparation by using SDS-polyacrylamide gel electrophoresis. A sample of [^{14}C]glucosamine-labeled E1 from virus grown in BHK cells was combined with [^{3}H]glucosamine-labeled E1 grown in chicken embryo cells. The mixture was digested with protease and chromatographed on Bio-Gel P-6, as described in Materials and Methods. The results are shown in the top panel of Fig. 4. A similar experiment comparing the glycopeptides of glycoprotein E2 from virus grown in these two hosts is shown in the bottom panel of Fig. 4. These results demonstrate that glyocprotein E1 from virus grown in BHK cells is deficient in glycopeptide S-4. In these figures, the S4 glycopeptides of both E1 and E2 are resolved into two peaks. Such resolution is not always achieved. However, the S4 glycopeptides always elute as broad peaks, possibly due to microheterogeneity.

The same conclusion was reached by examining the glycopeptides of pure E1 from virus grown in BHK cells and labeled with radioactive mannose. Purified virus labeled with [2-^{3}H]mannose was combined with purified virus labeled in BHK cells with [^{14}C]glucosamine, and then glycoproteins E1 and E2 were purified as described in Materials and Methods. Purified E1 was then digested with protease and chromatographed on Bio-Gel P-6 as before. The results are shown in the top panel of Fig. 5. A similar experiment, showing the mannose-labeled glycopeptides of glycoprotein E2, is shown in the bottom panel of Fig. 5. These experiments show that glycoprotein E1 from virus grown in BHK cells contains only small amounts of mannose-rich glycopeptide S4. Less than 20% of the total mannose and less than 10% of the total glucosamine counts in E1 are found in glycopeptide S4 (top panel, Fig. 5). These results are not simply due to an inefficiency in labeling S4, since it has proven difficult to isolate large quantities of this S4 during the preparation of large amounts of the remain-

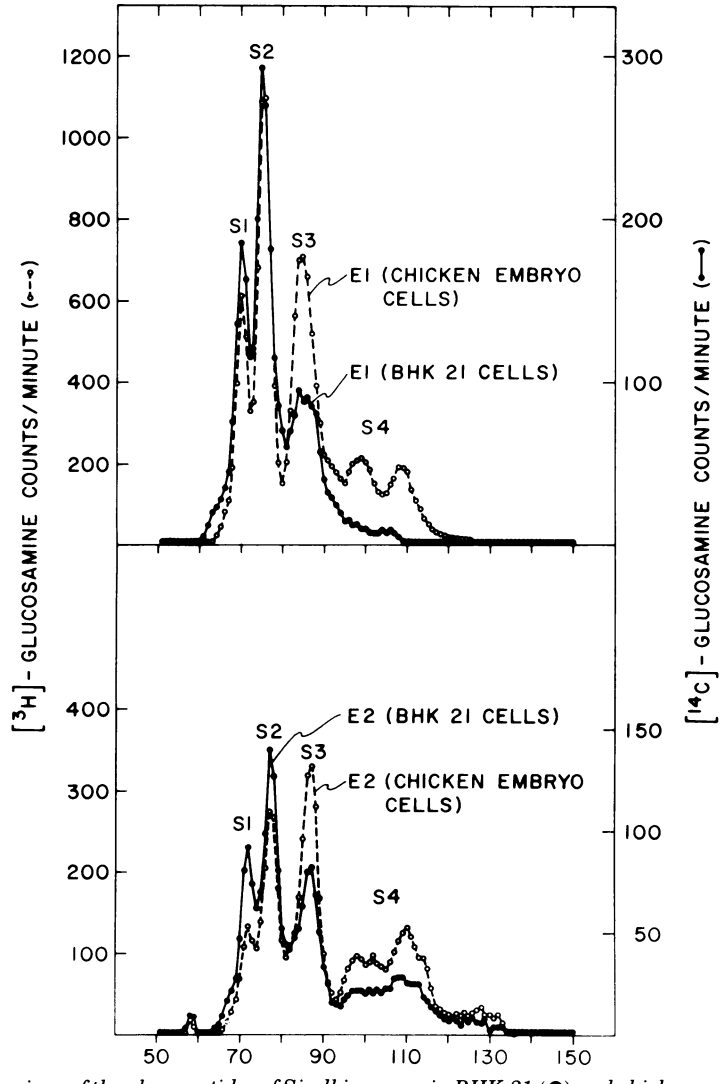

FIG. 4. *Comparison of the glycopeptides of Sindbis grown in BHK-21 (●) and chicken embryo (○) cells. The top panel shows Bio-Gel P-6 chromatography of the glycopeptides obtained by protease treatment of glycoprotein E1 purified from virus grown in the two hosts, and the bottom panel shows a similar comparison of the glycopeptides obtained from glycoprotein E2.*

ing E1 glycopeptides (Burke and Keegstra, unpublished observations). Glycoprotein E2 contains large amounts of mannose-rich glycopeptide S4 (bottom panel, Fig. 5).

To determine which of the glycopeptides of virus grown in BHK cells have A-type structures, virus was labeled in these cells with [^{14}C]galactose. Labeled glycoproteins E1 and E2 were prepared by SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. A sample of [^{14}C]galactose-labeled glycoprotein E1 was combined with [^{3}H]glucosamine-labeled E1, digested with protease, and chromatographed on Bio-Gel P-6 as described above. The results of this experiment are shown in the top panel of Fig. 6. A similar experiment using [^{14}C]galactose- and [^{3}H]glucosamine-labeled glycopeptides from glycoprotein E2 is shown in the bottom panel of Fig. 6. These experiments show that the S1, S2, and S3 glycopeptides from both E1 and E2 contain galactose, whereas the S4 glycopeptide on E2 lacks this sugar.

DISCUSSION

Procedures for the purification of the Sindbis virion proteins have been described, and chemical characterization of the proteins isolated

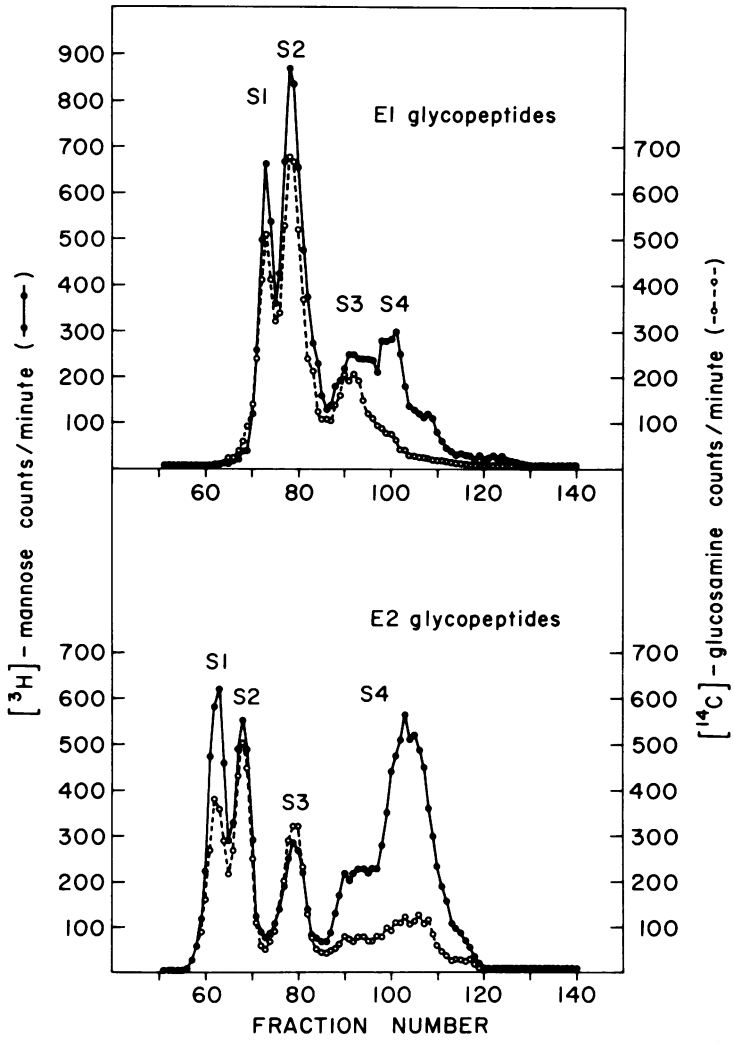

FIG. 5. *Mannose-labeled glycopeptides of Sindbis grown in BHK-21 cells. The top panel shows Bio-Gel P-6 chromatography of the glycopeptides obtained by protease treatment of glycoprotein E1 labeled with [2-^{3}H]mannose (●). [^{14}C]glucosamine-labeled E1 glycopeptides (○) were run as markers. The bottom panel is a similar experiment showing the mannose-labeled glycopeptides of glycoprotein E2.*

from Sindbis grown in chicken embryo and BHK cells have been presented. The data suggest that the oligosaccharide structures of glycoprotein E2 are very similar when virus is grown in the two hosts, but that glycoprotein E1 shows interesting, host-specific differences.

The carbohydrate compositions and the glycopeptide elution profiles of glycoprotein E2 preparations from virus grown in the two hosts are very similar (Table 2, Fig. 4). There are, however, some host-specific differences. When virus is grown in BHK cells, glycoprotein E2 contains more sialic acid (Table 2) than when the virus is grown in chicken embryo cells. Glycoprotein E2 from virus grown in both hosts contains four glycopeptide peaks (S1 through S4), and these glycopeptides have the same retention volumes regardless of the host cell (bottom panel, Fig. 4). In agreement with the increased sialic acid content when virus is grown in BHK cells, the glycopeptides of E2 from virus grown in that host have more of the largest A-type glycopeptides (S1 and S2) and less of glycopeptide S3 than when E2 is isolated from virus grown in chicken embryo cells.

Previous evidence (28) suggested that each molecule of glycoprotein E2 contained one A-type (S1, S2, or S3) and one B-type (S4) oligosaccharide chain. If this is true, and if the previously estimated molecular weights of the

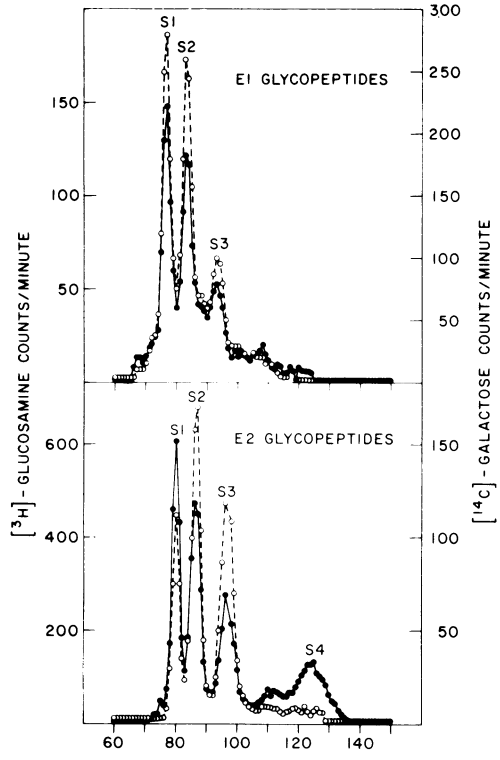

FIG. 6. *Galactose-labeled glycopeptides of Sindbis grown in BHK-21 cells. The top panel shows Bio-Gel P-6 chromatography of the glycopeptides obtained by protease treatment of glycoprotein E1 labeled with [1-^{14}C]galactose* (○). *[^{3}H]glucosamine-labeled E1 glycopeptides* (●) *were added as markers. The bottom panel is a similar experiment showing the galactose-labeled glycopeptides of glycoprotein E2.*

oligosaccharide chains (3) and of the glycoproteins (25) are correct, each molecule of glycoprotein E2 should contain approximately 4,000 daltons of carbohydrate. This corresponds to 80 μg of carbohydrate per mg of protein. The data in Table 2 are significantly below this value. There are several possible explanations for this low carbohydrate-to-protein ratio. First, it may be that glycoprotein E2 is incompletely glycosylated at either or both sites of carbohydrate attachment. Second, the estimated molecular weights of the glycopeptides or of glycoprotein E2 might be in error. Finally, these data may reflect an overestimation of the amount of protein in the samples used for analysis. There are several reasons for suspecting that the latter explanation may be correct. Glycoprotein E2 contains a higher percentage (4.4%) of tyrosyl residues than does the bovine serum albumin (3.21%; 24) used as an internal standard. In addition, the purification procedure makes use of Tris buffer and Triton X-100, both of which interfere with protein estimation by the Lowry procedure (22, 37). The Semliki forest virus glycoproteins bind considerable amounts of Triton X-100 (14), and our solvent precipitations might not have removed all of the bound detergent. The data in Table 2 and the bottom panel of Fig. 5 do suggest that glycoprotein E2 from virus grown in both hosts contains equal amounts of A- and B-type glycopeptides. However, the exact stoichiometry of the oligosaccharide chains must await more rigorous proof.

When Sindbis is grown in chicken embryo cells, glycoprotein E1 gives rise to four different glycopeptides after proteolysis and chromatography on Bio-Gel P-6 (28; Fig. 4). However, when Sindbis is grown in BHK cells, E1 contains much lower amounts of the mannose-rich glycopeptide S4 (top panels of Fig. 4 and 5). The remaining three A-type glycopeptides correspond in size to the S1, S2, and S3 peaks isolated from E1 of virus grown in chicken embryo cells. This deficiency in S4 is not merely due to an inefficiency in labeling that peak with glucosamine, since it is also not efficiently labeled with mannose (Fig. 5) and since only small amounts of this glycopeptide are found when glycopeptides are purified on a preparative scale (Burke and Keegstra, unpublished data). Since glycopeptide S4 contains only glucosamine and mannose (28; Burke and Keegstra, unpublished observations), the reduced amount of it in E1 from virus grown in BHK cells could account for the reduced mannose content of E1 in virus from that host (Table 2). The increased galactose content of E1 from virus grown in BHK cells is less readily explained. Since all galactose in these glycopeptides either is at the nonreducing terminus or is substituted by a single sialic acid residue (Burke and Keegstra, unpublished observation), it could be that glycopeptides S1, S2, and S3 contain progressively less galactose as well as progressively less sialic acid. As was true of glycoprotein E2, the carbohydrate contents of E1 from the two hosts is sufficient for one to two carbohydrate chains per protein molecule, but the exact stoichiometry must await further experiments.

The function of the B-type oligosaccharide structure found on glycoprotein E1 of virus from chicken embryo cells is obscure. It probably does not have a major role in the infective process, since virus particles grown in BHK cells are deficient in this structure but have a particle/PFU ratio similar to that of virus grown in chicken embryo cells (33). In fact, a great deal of the Sindbis virion carbohydrate can be removed or altered without loss of infectivity (27, 33).

The amino acid compositions of the Sindbis virion proteins (Table 1) have several interesting features. All three proteins are low in percentage of hydrophobic amino acids but are rich in proline. If, like the vesicular stomatitis virus and Semliki forest virus glycoproteins (11, 20, 35; R. H. Schloemer, Abstr. Annu. Meet. Am. Soc. Microbiol., 1975, S149, p. 238), the Sindbis glycoproteins have a hydrophobic tail, the remaining hydrophilic portion of these proteins would be further enriched in hydrophilic amino acids. The core protein is quite rich in lysine and arginine, but not as rich as histones (8). Both glycoproteins are rich in aspartate, glutamate, serine, and threonine. However, the percentages of aspartate and glutamate amidated are not known. Both glycoproteins have about the same total percentage of basic amino acids (Table 1). The fact that E2 has an isoelectric point of 9.0, whereas E1 has an isoelectric point of 6.0 (6), would suggest that E2 contains a much higher percentage of asparagine and glutamine than E1. Dalrymple et al. (6) found the isoelectric point of the core protein to be 3.0, but they suggested that their core protein probably contained RNA fragments. Our data, showing a high percentage of basic amino acids, would agree with that suggestion. The Sindbis glycoproteins have amino acid compositions very similar to the virion proteins purified from Semliki forest virus (12). This may indicate that the glycoproteins from these two viruses will have a great deal of homology, but that remains to be proven by sequence studies. The core proteins from the two viruses also have quite similar compositions, except for lysine and arginine (26). The total percentage of lysine plus arginine is similar in the core proteins from the two viruses. Such similarities between the compositions of these two viruses are not unexpected, since the viruses have somewhat similar structures and host ranges, and since alphaviruses display some antigenic cross-reactivity (4, 23, 26).

Several features of the virion protein purification scheme are interesting. First, it is necessary to perform the detergent solubilization of the envelope glycoproteins at a fairly high buffer concentration (0.2 M). If this is not done, glycoprotein E2 pellets with the nucleocapsid. This suggests either that there is a quite strong interaction between this envelope protein and the nucleocapsid or that glycoprotein E2 aggregates at low ionic strength. The latter seems unlikely as glycoprotein E2 cannot subsequently be solubilized by raising the ionic strength.

Brown and Smith (1) have reported that cells infected with the Sindbis mutant ts-23 fail both to hemadsorb and to align nucleocapsids along the plasma membrane. Since glycoprotein E1 is the apparent viral hemagglutinin (6), this suggests that glycoprotein E1 might traverse the membrane rather than E2, as is suggested by our observations. However, Brown and Smith (1) were not able to discriminate between two possible alternatives: (i) the temperature-sensitive alteration in glycoprotein E1 directly causes loss of both hemadsorption and ability of nucleocapsids to recognize viral proteins in the membrane; or (ii) the altered hemagglutinin prevents E2 (or PE2) from assuming the correct conformation or insertion into the membrane, thus blocking recognition of nucleocapsids. Although our evidence supports the conclusion that E2 interacts with the viral nucleocapsid, further experiments will be necessary to establish the nature of this interaction and the component(s) of the nucleocapsid involved. A second interesting feature of the purification scheme is the difficulty encountered in separating the two envelope proteins by ion-exchange chromatography. The fact that some E1 elutes with E2, and vice versa, probably means that there are long-lived aggregates of glycoproteins E1 and E2, even in the presence of detergent. This may indicate that glycoproteins E1 and E2 are tightly bound to one another in the viral envelope and in the membranes of infected cells. However, since reduction is not a necessary step in the purification of glycoproteins E1 and E2, it is unlikely that they are linked by disulfide bonds. Such tight binding could explain why regions of infected cell membranes containing E1 and E2 can exclude cellular proteins. Von-Bonsdorff and Harrison (36) have reported that the Sindbis glycoproteins occur in an icosahedral array on the virion surfaces. Such a regular array might also indicate strong, specific interactions between the two glycoproteins.

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