Carbohydrate Components of Influenza C Virions

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The carbohydrate components of influenza C virions grown in chicken kidney (CK) cells were analyzed by gel filtration following exhaustive digestion with Pronase. The [³H]glucosamine-labeled glycopeptides were larger and more heterogeneous than those of influenza A/WSN virions; three major size classes (G₁, G₂, and G₃) were resolved. Treatment with *Vibrio cholerae* neuraminidase caused a decrease in size of G₁ and G₂, along with release of about 16% of the ³H label. The released sugar components were identified as *N*-acetylneuraminic acid by thin-layer chromatography. Peak G₃ was highly labeled with [³H]mannose, whereas G₁ and G₂ contained lower levels of mannose. The three major viral glycoproteins gp88, gp65, and gp30 were isolated from sodium dodecyl sulfate-polyacrylamide gels, and their glycopeptide components were analyzed after Pronase digestion. The three size classes of glycopeptides were obtained from any of the three glycoproteins; however, the relative amounts of the three components varied among the glycoproteins. Host cell-derived components, which appear to be mucopolysaccharides and glycoproteins, were found associated with influenza C virions grown in CK cells. These components contained glycopeptides that were mainly of sizes similar to peak G₂ from influenza C virions. Previous studies have shown that influenza A/WSN virus grown in several cell types contained only two size classes of glycopeptides. Two size classes comparable to peaks G₂ and G₃ from influenza C virions were also observed in influenza A/WSN grown in CK cells. Thus the large G₁ glycopeptides appear to be characteristic of influenza C virions.

Influenza C viruses differ from types A and B influenza viruses in immunological properties as well as in the nature of their receptor-destroying enzymes. However, recent studies (6, 8, 12, 23) have shown that influenza C virions share most structural features of other orthomyxoviruses, including virions morphology, the presence of a segmented RNA genome, and the general types of major structural polypeptides. Three glycoprotein species have been observed in type C influenza virions, with approximate molecular weights of 88,000, 65,000 and 30,000 (6, 12). The two smaller glycoproteins resemble HA₁ and HA₂ of influenza A viruses in that they are linked by disulfide bonds (17). It is not certain whether gp88 is proteolytically cleaved to produce the two smaller glycoproteins, or whether it represents a distinct virus-coded polypeptide.

Evidence for a difference between the receptor-destroying enzyme of influenza C and other orthomyxoviruses was first obtained by Hirst (10), who showed that influenza A and B viruses destroyed their own receptors on erythrocytes without affecting the receptor for influenza C

virus. Conversely, influenza C virions eluted from erythrocytes with destruction of their receptors, whereas receptors for other ortho- and paramyxoviruses were not removed in the process (12, 17). Kendal (12) has obtained further evidence that the receptor-destroying enzyme of influenza C virions is not an α -neuraminidase, in that it did not liberate neuraminic acid from any of a series of neuraminidase substrates. Hemagglutination by influenza C virions is not inhibited by soluble glycoproteins that are active against influenza A viruses, whereas a rat serum glycoprotein is uniquely active against influenza C virions (22).

Enveloped viruses that lack a neuraminidase generally contain neuraminic acid as a component of their membrane glycoproteins and glycolipids. This may serve as a receptor for the hemagglutinin of influenza A virus, causing extensive formation of mixed aggregates of viruses, which results in hemagglutination inhibition (5). Evidence has been obtained for the presence of neuraminic acid in influenza C virions by the morphological finding of such mixed aggregates

(17) and by a hemagglutination-inhibition assay; pretreatment of type C virions with purified neuraminidase drastically reduced their capacity to inhibit hemagglutination by influenza A virus, indicating that neuraminic acid is involved (17, 21). In the present study, we have compared the types of carbohydrate components found in influenza C viral glycoproteins with those observed in influenza A viruses (19) by analyses of glycopeptides obtained after extensive Pronase digestion. We have found qualitative and quantitative differences between the oligosaccharide species of influenza A and C viral glycoproteins in virions grown in the same cell type. We have also obtained direct chemical evidence for the presence of neuraminic acid in influenza C virions.

MATERIALS AND METHODS

Virus and cells. Stocks of the Johannesburg/1/66 strain of influenza C virus were grown in embryonated eggs as described previously (17). Radiolabeled virus was grown in primary cultures of chick kidney (CK) cells as described elsewhere (T. Petri, H. Meier-Ewert, and R. W. Compans, FEMS Microbiol. Lett., in press). Stocks of influenza A/WSN virus were grown in MDBK cells as described by Choppin (3).

Radiolabeling and purification of virus. Monolayers of CK cells grown in 10-cm plastic petri dishes were washed with phosphate-buffered saline and infected with influenza C Johannesburg/1/66 stock virus at a multiplicity of infection of 0.01 PFU/cell. After adsorption for 1 h at 33°C, unadsorbed inoculum virus was removed, and 6 ml of Eagle minimal essential medium per dish, containing 2% calf serum and 5 μ Ci of [3 H]glucosamine or [3 H]mannose per ml, was added. At 48 h postinfection the culture medium was harvested and clarified by low-speed centrifugation. Virus was precipitated with 7.0% polyethylene glycol and 2.3% NaCl at 4°C for 90 min. The precipitate was pelleted by centrifugation at 4,000 rpm for 30 min, suspended in Dulbecco medium, and centrifuged in a 15 to 60% (wt/wt) sucrose gradient for 60 min at 27,000 rpm in a Spinco SW27 rotor. The virus band recovered from the gradient was diluted in 0.1 M Tris-hydrochloride (pH 7.2) and pelleted at 35,000 rpm for 45 min in a Spinco SW50.1 rotor. Virus was resuspended either in sample buffer (0.0625 M Tris-hydrochloride, pH 6.8, 1% sodium dodecyl sulfate [SDS], 1% β -mercaptoethanol, 10% glycerol, and 0.005% bromophenol blue) for gel electrophoresis or in 0.1 M Tris-hydrochloride (pH 8.0) containing 0.01 M CaCl₂ for glycopeptide analysis.

[3 H]glucosamine-labeled influenza A/WSN virus was grown in CK cells by infecting confluent monolayers at a multiplicity of infection of 2 to 5 PFU/cell. Unadsorbed virus was removed after 1 h, and 6 ml of Eagle minimal essential medium + 2% calf serum + 5 μ Ci of [3 H]glucosamine per ml was added per dish. At 24 h postinfection, virus was purified from culture medium as described above for influenza C virus. [14 C]glucosamine-labeled influenza A/WSN virus was

grown in MDBK cells as described previously (19).

Polyacrylamide gel electrophoresis. Polyacrylamide slab gel electrophoresis was performed in a 10% separation gel under a 3.6% stacking gel with a discontinuous buffer system as described by Laemmli (14). Virus samples were boiled for 1 min before electrophoresis. After the run the gels were either prepared for fluorography according to Bonner and Laskey (1) or sliced and eluted for the isolation of the glycoproteins as described by Nakamura and Compans (19).

Isolation of viral glycoproteins. Influenza C virions grown in CK cells labeled with [3 H]glucosamine were purified, and the viral glycoproteins were separated by SDS-polyacrylamide gel electrophoresis. Viral glycoproteins were isolated from gels according to the procedures described previously (19). To isolate HA₁ and HA₂ from influenza A/WSN virus, the virus was grown in CK cells labeled with [3 H]glucosamine. The NA glycoproteins, which have electrophoretic mobility similar to HA₁, were removed by treatment of purified virions with trypsin as described previously (19). HA₁ and HA₂ were isolated after SDS-polyacrylamide gel electrophoresis of the trypsin-treated virions.

Glycopeptide analysis. Either purified virions or isolated glycoproteins suspended in 0.1 M Tris-hydrochloride (pH 8.0) containing 0.01 M CaCl₂ were extensively digested with Pronase as described previously (18). The resultant glycopeptides were analyzed by gel filtration on a column of Bio-Gel P-6 (100/200 mesh, 1.0 by 115 cm) equilibrated with 0.1 M Tris-hydrochloride (pH 8.0) as described previously (18).

Neuraminidase treatment of glycopeptides. The fractions containing glycopeptides to be tested, which were obtained after gel filtration of Pronase-digested glycopeptides, were pooled, lyophilized, and dissolved in deionized water. They were desalted utilizing a column of Bio-Gel P-2 (50/100 mesh, 1.5 by 25 cm) and lyophilized. The glycopeptides were dissolved in 500 μ l of 0.1 M sodium acetate buffer (pH 5.5). After addition of 50 μ l of neuraminidase (500 U/ml), the mixture was incubated at 37°C. An additional 50 μ l of neuraminidase was added at 12 h of incubation, and the reaction was terminated at 24 h by boiling for 2 min.

Thin-layer chromatography. Samples of 1 to 10 μ l were applied to silica gel-coated glass plates (0.25-mm thickness) and developed with *n*-butanol-acetic acid-water (2:1:1). Standard sugars were visualized with anisaldehyde (15). To detect labeled sugars, fractions of 2-mm length were scraped into vials for determination of radioactivity in a liquid scintillation counter.

Chemicals and isotopes. Silica gel-coated plates were obtained from E. Merck (Darmstadt, West Germany). Pronase (*Streptomyces griseus* protease), B grade, was obtained from Calbiochem (La Jolla, Calif.). Neuraminidase isolated from culture filtrates of *Vibrio cholerae* was obtained from Behring Diagnostics (Somerville, N.J.). D-[6- 3 H]glucosamine (6.3 Ci/mmol) and D-[1- 14 C]glucosamine (58.2 mCi/mmol) were obtained from ICN Pharmaceuticals (Irvine, Calif.). D-[2- 3 H]mannose (2 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, Ill.).

RESULTS

Glycoproteins of influenza C virions grown in CK cells. Previous results (6, 12) have shown that influenza C virus grown by amniotic inoculation of embryonated chicken eggs contains three distinct sizes of glycoproteins, which are designated gp88, gp65, and gp30 on the basis of their molecular weights. In contrast, only the largest glycoprotein, gp88, was detectable in virions grown in primary chicken embryo fibroblast cells (6).

SDS-polyacrylamide gel electrophoresis of purified virions grown in primary CK cells labeled with [^3H]glucosamine revealed the presence of three glycoproteins with molecular weights similar to those of virions grown in embryonated eggs (Fig. 1 and 4). Of the [^3H]glucosamine label incorporated into viral glycoproteins, about 48, 31, and 21% was found in gp88, gp65, and gp30, respectively. In addition to these three glycoproteins, as much as 75% of the label recovered in association with purified virus was distributed into components with much lower electrophoretic mobilities than any viral glycoproteins described above. When virus was grown in cells prelabeled with [^3H]glucosamine, two components near the top of the gel were selectively labeled without significant labeling of gp88, gp65, or gp30 (Fig. 1A). These two bands were also heavily labeled with [^3H]glucosamine, in addition to three bands corresponding to viral glycoproteins, when virions were labeled as described in Materials and Methods (Fig. 1B). Both of the bands with low electrophoretic mobilities were also detectable in gel patterns of uninfected primary CK cells labeled with [^3H]glucosamine (data not shown), indicating that these are host cell-derived components.

Glycopeptides of influenza C virions. To obtain information about the oligosaccharide components of influenza C viral glycoproteins, purified virus grown in CK cells and labeled with [^3H]glucosamine was extensively digested with Pronase and co-chromatographed on Bio-Gel P-6 with the glycopeptides from influenza A/WSN virus grown in MDBK cells labeled with [^{14}C]glucosamine. A large amount of ^3H label (about 35%) eluted around the void volume (Fig. 2A). Our previous studies with WSN virions indicated that host cell-derived mucopolysaccharides are recovered in these fractions (20). Most of the remaining ^3H label eluted heterogeneously in the fractions in which glycopeptides from WSN virus were detected. Because of previous evidence that influenza C virions contain neuraminic acid (17, 21), it was of interest to exam-

FIG. 1. Fluorogram of [^3H]glucosamine-labeled purified influenza C Johannesburg 1/66 virus proteins separated on a polyacrylamide gel as described in the text. (A) Label ($5\ \mu\text{Ci}/\text{ml}$) was present from 10 to 2 h prior to infection. (B) Label ($5\ \mu\text{Ci}/\text{ml}$) was present from 1 to 48 h postinfection. Virus from both preparations was purified, run in parallel on a slab gel, and analyzed by fluorography. The positions of the virion glycoproteins are indicated by arrows.

ine the effect of neuraminidase treatment on the elution profile of influenza C glycopeptides. The fractions containing glycopeptides were pooled and divided into two portions. One was treated with neuraminidase as described in Materials and Methods, and the other was untreated; the samples were analyzed by rechromatography on Bio-Gel P-6. At least three peaks of ^3H label were resolved in the control (Fig. 2B). The main peak of ^3H label (peak G_2) coeluted with that of ^{14}C label, which corresponds to the complex galactose-containing type I glycopeptides of WSN virus. A small peak of ^3H label (peak G_3) was found in the shoulder fractions of ^{14}C label, which corresponds to mannose-rich type II glycopeptides of WSN virus (19). In addition to these two peaks, another peak was detectable around fraction 35 and designated peak G_1 . The sizes of glycopeptides G_1 and G_2 of influenza C virus were apparently decreased after neuraminidase treatment (Fig. 2C), whereas that of peak G_3 was not, suggesting that neuraminic acid residues are present in glycopeptides G_1 and G_2 .

FIG. 2. Glycopeptides from influenza C virions grown in CK cells labeled with [^3H]glucosamine. Influenza C virus was grown in CK cells in the presence of [^3H]glucosamine and purified as described in the text. The purified virions were mixed with influenza A/WSN virions grown in MDBK cells labeled with [^{14}C]glucosamine. The mixture was extensively digested with Pronase, and the resultant glycopeptides were analyzed by gel filtration on Bio-Gel P-6 (A). Fractions 35 to 65 were pooled from (A) and divided into two portions. One was treated with neuraminidase, and the other remained untreated and served as control. The control (B) and neuraminidase-treated (C) glycopeptides were rechromatographed on Bio-Gel P-6. The three size classes designated as G_1 , G_2 , and G_3 are indicated in (B).

Figure 2C also shows that a peak containing about 16% of the ^3H label appeared in fractions 70 to 75 after neuraminidase treatment. No significant amount of ^{14}C label was detectable in these fractions. This peak was similar to the elution profile of neuraminic acid rather than free [^3H]glucosamine (data not shown). The ^3H -labeled components released from virions by neuraminidase treatment were identified as N-

acetylneuraminic acid by thin-layer chromatography on silica gel (Fig. 3). In control samples not treated with neuraminidase, all ^3H label was recovered at the origin of the chromatogram.

Glycopeptides of isolated viral glycoproteins. Since there is a large amount of host-derived components that can be labeled with [^3H]glucosamine in influenza C virus preparations, analyses of glycopeptides were also carried out with isolated viral glycoproteins. The three glycoproteins were isolated from influenza C virus labeled with [^3H]glucosamine according to the procedures described in Materials and Methods. A representative gel pattern obtained in such experiments is shown in Fig. 4. Each isolated glycoprotein was mixed with WSN virions grown in MDBK cells labeled with [^{14}C]glucosamine, digested with Pronase, and applied on a column of Bio-Gel P-6. Three distinct sizes of glycopeptides were clearly observed with any of three viral glycoproteins (Fig. 5A, C, and E). It is apparent, however, that the relative amounts of the three size classes of glycopeptides varied

FIG. 3. Thin-layer chromatography of ^3H -labeled sugar released from neuraminidase-treated influenza C virions. Purified influenza C/JHB/1/66 virions labeled with [^3H]glucosamine were suspended in 10 μl of 0.01 M phosphate buffer (pH 7.2) containing 0.5 U of *V. cholerae* neuraminidase (Behringwerke, West Germany). After incubation for 90 min at 37°C , virus particles were sedimented in an SW50.1 rotor at 33,000 rpm for 30 min. The supernatant was transferred to a silica gel-coated plate and developed as described in the text. The arrows indicate the position of standard sugars that were run in parallel: 1, N-acetylneuraminic acid; 2, galactosamine; 3, glucosamine; 4, galactose; 5, glucose; 6, N-acetylgalactosamine; 7, mannose; 8, N-acetylglucosamine; 9, fucose.

among the viral glycoproteins. When the fractions containing glycopeptides were pooled, treated with neuraminidase, and then rechromatographed on Bio-Gel P-6, the sizes of glycopeptides G_1 and G_2 were decreased for each glycoprotein although glycopeptide G_2 was not clearly resolved with gp30 (Fig. 5B, D, and E). In contrast, glycopeptide G_3 appeared not to be affected by neuraminidase treatment in any of the three glycoproteins. Further, peaks which probably represent free neuraminic acid were produced by neuraminidase treatment of each glycoprotein. These results indicate that gp88, gp65, and gp30 all contain neuraminic acid residues in their carbohydrate side chains, and that neuraminic acid is associated with the two larger size classes of glycopeptides, G_1 and G_2 , but not with the smallest one, G_3 .

Glycopeptides of host cell components in influenza C preparations. The results shown in Fig. 2 indicated that purified influenza C virions contain glycopeptide G_2 in much higher amounts than either glycopeptide G_1 or G_3 . This observation cannot be explained if viral glycoproteins are the only source of glycopeptides in the preparation of purified virus (compare Fig. 2 with Fig. 5). It therefore appears that host cell-derived components contributed to the glyco-

FIG. 4. SDS-polyacrylamide gel electrophoresis pattern of glycoproteins of influenza C virions grown in CK cells labeled with [3 H]glucosamine.

FIG. 5. Glycopeptides associated with glycoproteins isolated from influenza C virions. Influenza C virus was grown in CK cells labeled with [3 H]glucosamine. Viral glycoproteins gp88, gp65, and gp30 were isolated after SDS-polyacrylamide gel electrophoresis of purified virions as described in the text. After Pronase digestion of each isolated viral glycoprotein, the resultant glycopeptides were applied to a column of Bio-Gel P-6. The fractions containing glycopeptides were pooled, treated with neuraminidase, and rechromatographed. Glycopeptides from gp88, (A) before and (B) after neuraminidase treatment. Glycopeptides from gp65, (C) before and (D) after neuraminidase treatment. Glycopeptides from gp30, (E) before and (F) after neuraminidase treatment. Dashed lines show the elution profiles of [14 C]glucosamine-labeled glycopeptides from influenza A/WSN virions grown in MDBK cells.

peptides shown in Fig. 2. To examine the glycopeptides of the host components, they were isolated after SDS-polyacrylamide gel electrophoresis of purified influenza C virus labeled with [^3H]glucosamine. After extensive digestion with Pronase, the digested materials were applied on a Bio-Gel P-6 column (Fig. 6A). About 50% of the label was recovered around the void volume, which probably represents mucopolysaccharides. Most other label was found in the fractions where glycopeptide G₂ of influenza C viral glycoproteins eluted. This result does not conclusively show whether these host components contain glycopeptides with sizes similar to either glycopeptide G₁ or G₃ of influenza C viral glycoproteins. To determine if neuraminic acid residues are present in the glycopeptides from host components, the fractions from Fig. 6A containing glycopeptides were pooled, treated with neuraminidase, and then rechromatographed on Bio-Gel P-6. The result (Fig. 6B) demonstrates that treatment of the glycopeptides with neuraminidase caused a shift of the main peak to lower-molecular-weight fractions as well as the appearance of a new peak in fractions 65 to 75, indicating the presence of neuraminic acid residues in oligosaccharide chains associated with host components. A shoulder observed in fractions 49 to 60 might be due to the presence of glycopeptides with sizes similar to glycopeptide G₃ of influenza C virus. It should be noted that the glycopeptides of host components do not contain a peak corresponding to glycopeptide G₁ of influenza C viral glycoproteins.

Glycopeptides of influenza C virions labeled with [^3H]mannose. To further characterize the glycopeptides of influenza C virions, we examined the incorporation of various radioactive sugar precursors into the viral glycoproteins. Either [^3H]fucose or [^3H]galactose was incorporated at very low levels into influenza C virions grown in CK cells, and a large part of the label recovered in purified virions was found to be associated with the host components described above. Although the incorporation of [^3H]mannose into influenza C virions was also low compared with [^3H]glucosamine, the relative amount of label recovered in host components was relatively low, about 35% of total counts associated with purified virions. Figure 7 shows the elution profile of [^3H]mannose-labeled glycopeptides obtained after Pronase digestion of influenza C virions grown in CK cells. The main peak of glycopeptides corresponds to glycopeptide G₃, and heterogeneous shoulders are seen in fractions where glycopeptides G₁ and G₂ elute. These results together with those obtained

FIG. 6. Glycopeptides from host cell-derived components in purified influenza C virus preparations. Influenza C virions were purified from culture fluids of infected CK cells labeled with [^3H]glucosamine, and then subjected to SDS-polyacrylamide gel electrophoresis. Host components were isolated from fractions of the gel and mixed with [^{14}C]glucosamine-labeled glycopeptides from influenza A/WSN virions grown in MDBK cells. After extensive digestion with Pronase, the resultant glycopeptides were analyzed by gel filtration on Bio-Gel P-6 (A). Fractions 35 to 65 were collected from (A), treated with neuraminidase, and rechromatographed (B).

with [^3H]glucosamine suggest that although all three size classes of influenza C glycopeptides contain mannose as well as glucosamine, the sugar composition of each is distinct; glycopeptides in peak G₃ are significantly richer in mannose than glycopeptide G₁ or G₂.

Glycopeptides of influenza A/WSN virions grown in CK cells. The results described above revealed the presence of three size classes of glycopeptides in influenza C viral glycoproteins. On the other hand, previous studies from our laboratory have shown that influenza A/WSN virions grown in four different cell types contain only two size classes of glycopeptides, which are comparable in size to glycopeptides G₂ and G₃ of influenza C virus, although CK cells were not examined (19). To determine if the incorporation of the largest glycopeptides (G₁) is dependent on influenza C viral glycoproteins or on the host cell, the WSN strain was grown in

FIG. 7. Glycopeptides from influenza C virions labeled with [^3H]mannose. Influenza C virions, grown in CK cells in the presence of [^3H]mannose, were purified and digested with Pronase. The resultant glycopeptides were co-chromatographed with [^{14}C]-glucosamine-labeled glycopeptides from A/WSN virions grown in MDBK cells.

CK cells labeled with [^3H]glucosamine. The glycopeptides obtained after Pronase digestion of the purified virus were analyzed on Bio-Gel P-6. The result (Fig. 8) indicates that, as has been observed with other host cell types (19), WSN virus grown in CK contains only two size classes of glycopeptides and apparently lacks large glycopeptides comparable to G_1 of influenza C virus. Figures 8B and C show that the large glycopeptides were found in neither isolated HA_1 nor HA_2 of WSN virus grown in CK cells, and that HA_1 contains two size classes of glycopeptides whereas HA_2 contains essentially only type I glycopeptides.

DISCUSSION

The presence of two types of oligosaccharide chains has been found in various secreted and membrane glycoproteins (11, 16, 19, 24, 25). One is a complex type, composed of glucosamine, mannose, galactose, and fucose; sialic acid may or may not be contained in these oligosaccharides. The other is a high-mannose type, which is rich in mannose and contains glucosamine, but lacks galactose and fucose. We have observed three size classes of glycopeptides in each of the glycoproteins of influenza C virions. The smallest class, peak G_3 , had a size similar to type II glycopeptides of influenza A/WSN virus and was labeled with [^3H]mannose to a much higher extent than either glycopeptides G_1 or G_2 , suggesting that these glycopeptides are similar to the high-mannose type of glycopeptides described in other systems. The similarity in size between neuraminidase-treated glycopeptide G_2 of influenza C virus and type I glycopeptides of influenza A/WSN virus suggests that G_2 may be a complex type of glycopeptide. However, pre-

vious studies (4, 19, 24) have indicated that influenza A viruses contain only two size classes of glycopeptides, and that glycopeptides as large as G_1 of influenza C virions were not detected. We have recently compared the glycopeptides of the hemagglutinin from various antigenic subtypes of influenza A viruses (Nakamura and Compans, *Virology*, in press), and in no instance were glycopeptides as large as G_1 observed. Although several size classes of glycopeptides have been found in either vesicular stomatitis or Sindbis virus, only one or two classes of glycopeptides were detectable after treatment with neuraminidase (9, 25). In addition, the present studies showed that neither influenza A/WSN virions grown in CK cells nor host components associ-

FIG. 8. Glycopeptides from influenza A/WSN virus grown in CK cells. Purified influenza A/WSN virions labeled with [^3H]glucosamine were digested with Pronase and co-chromatographed with [^{14}C]-glucosamine-labeled glycopeptides from virions grown in MDBK cells (A). HA_1 and HA_2 were isolated from influenza A/WSN virions grown in CK cells labeled with [^3H]glucosamine according to procedures described previously (19). The glycopeptides prepared from HA_1 (B) or HA_2 (C) were also analyzed by gel filtration on Bio-Gel P-6. The type I and type II glycopeptides are indicated by arrows.

ated with purified influenza C virions contain glycopeptides comparable in size to G_1 . It therefore appears that the presence of glycopeptides as large as G_1 is unusual and may be specific for influenza C virus. Since neuraminic acid residues are present in this class of glycopeptides, and it has been generally found that neuraminic acid is associated with complex-type glycopeptides but not with the high-mannose type (13), G_1 may be structurally similar to complex rather than high-mannose-type glycopeptides. However, further studies are required to determine the structure as well as the mechanism of synthesis of G_1 glycopeptides.

The presence of neuraminic acid residues in influenza C viral glycoproteins has been suggested by findings that extensive formation of aggregates occurs when influenza C virus is mixed with influenza A virus (17), and that influenza C virus has a capacity to inhibit the hemagglutination by influenza A virus, which is reduced by pretreatment of influenza C virus with neuraminidase (17, 21). These two phenomena appear to be caused by the same mechanism, aggregate formation induced by interaction of influenza A virus with C virus. However, the basis for interaction between influenza A and C viruses is not fully understood yet. In particular, the present findings that host components were recovered in association with purified influenza C preparations, and that they contain neuraminic acid residues in their oligosaccharide moieties, raise the possibility that these components may have functioned as receptors for influenza A virus hemagglutinin and caused the aggregate formation in the mixture of influenza A and C viruses.

Three size classes of glycopeptides were found in any of the three influenza C viral glycoproteins. The ratios of the glycopeptides, however, were quite distinct among the three glycoproteins. Quantitative or qualitative differences in carbohydrate side chains among viral glycoproteins have been demonstrated with influenza A (19, 24) and Sindbis viruses (2). The available information suggests that viral glycoproteins are glycosylated by host cell transferases. These observations, however, suggest that carbohydrate side chains of viral glycoproteins may be specified by the nature of viral glycoproteins in addition to host cellular transferases.

The presence of host cell-derived mucopolysaccharides in highly purified virion preparations has been previously demonstrated with influenza A/WSN virus (7). These mucopolysaccharides remain near the origin of gels after SDS-polyacrylamide gel electrophoresis (7), and they elute around the void volume after gel

filtration of Pronase digests (20). Similarly, it seems likely that the upper band of host components in the SDS-polyacrylamide gels of influenza C virions may correspond to mucopolysaccharides. The lower band appears to be a glycoprotein, since it was labeled with [35 S]methionine as well as [3 H]glucosamine (data not shown). It appears unlikely that this component is due to the contamination of purified virion preparation by cellular membrane structures, since cytopathic effects were minimal in CK cells infected with influenza C virus, and membrane structures other than virions were not detectable by electron microscopy of purified influenza C preparations. This glycoprotein might be a secreted glycoprotein or a glycoprotein released from CK cells as a result of turnover of membrane proteins, and might be found in virus preparations because of high affinity for the virions. If this is the case, further analysis of this component may reveal information about the nature of the receptor for influenza C virus.

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