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Oligosaccharides of the Glycoprotein of Rabies Virus

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The number of oligosaccharide side chains on rabies virus glycoprotein (G-protein) was investigated. Analysis of glycopeptides obtained by protease digestion of desialated G-protein revealed three discrete glycopeptides. Comparison of the protease digestion products from desialated and from untreated G-protein indicated a heterogeneity among the glycopeptides in the sialic acid content. Two major tryptic glycopeptides were isolated from desialated rabies virus G-protein and analyzed after protease digestion; one contained two oligosaccharide side chains and the other contained a single oligosaccharide side chain.

The rabies virus envelope contains a single glycoprotein (G-protein) (14) that consititutes the basic unit of the surface projections of the virus (B. Dietzschold, J. M. Cox, and L. G. Schneider, submitted for publication). This G-protein, which has been isolated in a biologically active and pure form, is the only virion antigen that induces and reacts with rabies virus-neutralizing antibodies (1, 21). Furthermore, vaccination experiments with purified G-protein have been shown to protect mice from challenge infection with rabies virus (1, 21). Chemical analysis has shown that 2.9% of the total mass of rabies virus grown in baby hamster kidney (BHK) cells consists of protein-bound carbohydrate (12). Mannose, galactose, *N*-acetyl-glucosamine, and neuraminic acid are the major constituents of the rabies virus G-protein, with fucose present in a smaller amount (12).

The present report analyzes glycopeptides obtained by trypsin or protease digestion of rabies virus G-protein. The data obtained indicate that rabies virus G-protein contains three oligosaccharide side chains.

MATERIALS AND METHODS

Cells and virus. The ERA strain of rabies virus was grown in roller cultures with BHK-21 S13 cells (18); culture conditions, virus assays, and purification techniques have been described (20). To prepare carbohydrate-labeled virus, BHK cells were infected with ERA virus; 48 h after infection, the culture medium was replaced by minimal essential medium supplemented with 0.3% bovine serum albumin and radioactive sugars at the following final activities per ml: 30 μCi of D-*N*-[6-^{3}H]glucosamine-hydrochloride (20.7 Ci/mmol); 30 μCi of D-*N*-[1-^{3}H]mannose (13.2 Ci/mmol); 30 μCi of D-*N*-[1-^{3}H]-galactose (14.2 Ci/mmol), and 30 μCi of L-[1,5,6-^{3}H]fucose (2.4 Ci/mmol). The cells were then incubated at 37°C for 24 h.

Isolation of the virus G-protein. Triton X-100 was added to a suspension of purified rabies virus to a final concentration of 2%. The mixture was kept at room temperature for 20 min and then chilled in an ice bath and centrifuged in a Beckman SW 50.1 rotor at 45,000 rpm for 60 min at 4°C. The supernatant containing the G-protein was dialyzed against 1,000 volumes of 0.01 M NH_4HCO_3 (pH 8.1) and lyophilized. The lyophilized material was suspended in a small volume of distilled water and precipitated with 15 volumes of cold acetone. To remove glycolipids, the precipitate was washed once with cold ethanol and twice with boiling ethanol (5), suspended in 0.01 M NH_4HCO_3 (pH 8.1), and lyophilized.

PAGE and isoelectric focusing. Polyacrylamide gel electrophoresis (PAGE) of viral polypeptides was carried out in a discontinuous polyacrylamide gel system having a 4% (wt/vol) stacking gel and a 10% (wt/vol) resolving gel as described by Laemmli (8). Purified virus or purified G-protein was precipitated with 5 volumes of ethanol and solubilized with a small volume of 1% (wt/vol) sodium dodecyl sulfate (SDS) and 1% (wt/vol) 2-mercaptoethanol. Before application to the SDS gels, samples were incubated for 1 min in a boiling-water bath. After electrophoresis, the gels were sliced into 1-mm sections, and individual slices were treated for 18 h with Protosol (New England Nuclear) at 37°C before adding scintillation fluid Omnifluor (New England Nuclear) toluene cocktail. Isoelectric focusing was carried out in an LKB 110-ml column as described previously (Dietzschold, Cox, and Schneider, submitted for publication).

Removal of sialic acid. (i) Mild acid hydrolysis. G-protein or glycopeptides were dissolved in 0.1 N H_2SO_4, incubated for 30 min at 80°C (19), cooled, and then neutralized with 1 M Tris base. The samples were then dialyzed against 1,000 volumes of 0.01 M NH_4HCO_3 (pH 8.1) and lyophilized.

(ii) Neuraminidase digestion. Labeled rabies virus G-protein was dialyzed extensively against 0.05 M sodium acetate buffer (pH 5.5)-0.15 M NaCl-0.005 M $CaCl_2$. *Vibrio comma* neuraminidase (A grade, BehringWerke) was added to a final concentration of

2 μg/ml, and the solution was incubated at 37°C for 1 h.

Preparation of tryptic glycopeptides. Tryptic digestion of rabies virus G-protein was carried out essentially as described by Cooper et al. (4). Isolated G-protein was suspended in NTE buffer (0.1 M NaCl-0.05 M Tris-hydrochloride [pH 7.5]-0.001 M EDTA), 0.5 mg of bovine serum albumin was added, and the suspension was heated at 100°C for 1 min with 1/1,000 volume of a mixture of 2-hydroxyethyldisulfide and 2-mercaptoethanol (50:1) and then dialyzed against 1,000 volumes of 0.05 M NH_4HCO_3 (pH 8.1). Freshly prepared 1% tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin solution (0.05 ml; Worthington) in 0.001 M HCl was added per ml of G-protein solution. After incubation at 37°C for 2.5 h, another 0.05 ml of 1% trypsin was added, and the incubation was continued for another 2.5 h. The sample was then lyophilized. The products of this digestion are referred to as tryptic glycopeptides.

Protease digestion of the G-protein and tryptic glycopeptides. Isolated G-protein or tryptic glycopeptides were suspended in 0.1 M NH_4HCO_3, pH 8.0. One drop of toluene was added, and the suspension was incubated with 0.1% self-digested protease from streptomyces (repurified, TypVI, Sigma) at 37°C for 4 days with daily addition of 0.1% protease so that the final protease concentration was 0.4% The samples were then lyophilized. The products of this digestion are referred to as "protease glycopeptides."

Analysis of glycopeptides by DEAE-cellulose chromatography. Tryptic glycopeptides or glycopeptides obtained by protease digestion were solubilized in a small volume of 0.05 M Tris-hydrochloride (pH 8.0) and applied to a column (90 by 0.9 cm) of DEAE-cellulose equilibrated with 0.05 M Tris-hydrochloride (pH 8.0) and eluted with a 400-ml linear gradient of 0 to 0.3 M NaCl in 0.05 M Tris, pH 8.0. The flow rate was approximately 80 ml/h, and 2-ml fractions were collected. A sample of each fraction was assayed for radioactivity. In some cases, the peak fractions were pooled, dialyzed against 0.01 M NH_4HCO_3 (pH 8.1), and lyophilized pending further analysis.

Analysis of glycopeptides on Dowex AG 50 × 4. Chromatography on Dowex AG 50 × 4 was performed at 50°C as described by Moore (10) on a column (25 by 1 cm). The column was equilibrated with 0.2 N citric acid, and the sample was eluted with a 300-ml linear gradient of 0.2 M citric acid to 0.5 M Na citrate, pH 6.0.

Gel filtration chromatography. Glycopeptides obtained by protease digestion or trypsin digestion were dissolved in a solution of 0.15 M Tris acetate buffer (pH 7.8) and 0.1% SDS; Blue Dextran 2000 (Pharmacia) was added to define the void volume. Glycopeptide samples (1 ml) were layered on the top of a Bio-Gel P6 (100 to 200 mesh, BioRad) column (0.9 by 80 cm) equilibrated with 0.15 M Tris-acetate (pH 7.8) containing 0.1% SDS and then eluted with the same buffer. Fractions (1 ml) were collected and counted directly in Formula 963 (New England Nuclear) scintillation fluid. The Bio-Gel P6 column was calibrated by determining the elution volume of the following glycopeptides of known molecular weights: thyroglobulin glycopeptide (molecular weight 4,100) (15), fetuin glycopeptide (molecular weight 4,400) (2), and ovalbumin glycopeptide (molecular weight 1,500) (17). Furthermore, raffinose and stachyose were used as standards. Ovalbumin, raffinose, and stachyose were detected by the phenol-sulfuric acid assay (16), and thyroglobulin was detected by the thiobarbituric acid assay (19).

RESULTS

Electrophoretic analysis of the rabies virus G-protein. Figure 1a shows the SDS-PAGE profiles of radiolabeled polypeptides from ra-

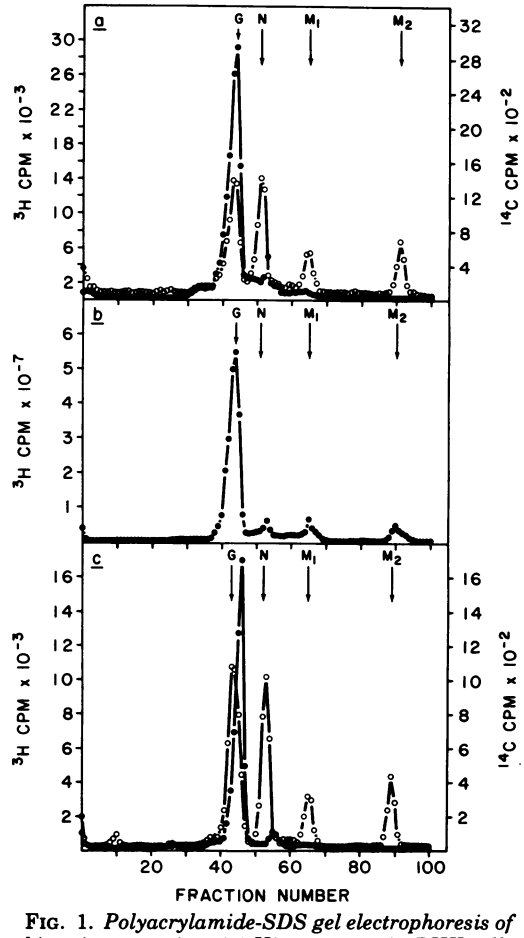

FIG. 1. *Polyacrylamide-SDS gel electrophoresis of rabies virus proteins. (a) Virus grown in BHK cells and labeled with [³H]glucosamine (●) and ¹⁴C-labeled-amino acids (○) was purified, and polypeptides were analyzed in 10% SDS-10% polyacrylamide gel as described in the text. (b) Rabies virus glycoprotein isolated from [³H]leucine-labeled virus as described in the text. (c) Co-electrophoresis on 10% SDS gel of ¹⁴C-labeled-amino acid-labeled rabies virus polypeptides (○) with neuraminidase-treated [³H]glucosamine-labeled G-protein (●) isolated as above.*

bies virus grown in the presence of either ^{14}C-labeled amino acids or [^{3}H]glucosamine. A single major viral G-protein and a smaller minor G-protein were labeled with [^{3}H]glucosamine. The minor G-protein, which migrated slightly faster than the nucleocapsid protein, is a degradation product of the major viral G-protein since this component accumulates after storage of the virus at 25°C (unpublished data). Figure 1b is an electropherogram on 10% SDS gel of [^{3}H]leucine-labeled G-protein purified as described above. Only small amounts of M_1 and M_2 protein can be seen and no nucleocapsid protein was detected by comigration with marker N. The minor peak near N corresponded with the degradation product of G.

Purified [^{3}H]glucosamine-labeled G-protein was treated with neuraminidase and compared with untreated ^{14}C-labeled amino acid-labeled rabies virus proteins by co-electrophoresis. Figure 1c reveals that the desialated glycoprotein migrated faster than the untreated control. Furthermore, isoelectric focusing analysis revealed that neuraminidase treatment shifted the isoelectric point of the viral G-protein from pH 7.0 to pH 7.5 (data not shown). This increase in the isoelectric point and the increased electrophoretic mobility of the neuraminidase-treated G-protein as compared with that of the untreated G-protein indicate the presence of sialic acid in the rabies virus G-protein.

Gel filtration of carbohydrate glycopeptides obtained by protease digestion on Bio-Gel P6. Isolated rabies virus G-protein was exhaustively digested with protease and analyzed on Bio-Gel P6. When the protease digestion product of sialated G-protein was chromatographed on Bio-Gel P6, two major components were obtained (Fig. 2a). The apparent molecular weights of these two glycopeptides were 4,300 and 3,700, as estimated by the method of Burge and Huang (2). After mild acid hydrolysis to remove the sialic acid residues from these glycopeptides, three components (Fig. 2b) were observed with apparent molecular weights of 3,400, 2,800, and 2,500. Neuraminidase treatment of the G-protein produced the same result (data not shown) as that obtained by mild acid hydrolysis. The observation that after removal of sialic acid all of the glycopeptides were smaller than the sialated glycopeptides indicates that the majority of the glycopeptide chains of the viral G-protein contained terminal sialic acid residues.

Bio-Gel P6 chromatography revealed that "protease glycopeptides" from rabies virus grown at 33°C were larger than similarly derived glycopeptides from rabies virus grown at 37°C. After removal of sialic acid, however, the corresponding glycopeptides from rabies virus grown at 33 and 37°C were exactly the same size (data not shown). These results suggest that the amount of sialic acid per glycopeptide is variable and that the G-protein in rabies virus grown at high temperature contains less sialic acid than that grown at lower temperatures.

To study the carbohydrate composition of the three glycopeptides derived by protease digestion and removal of sialic acid, preparations of desialated G-protein labeled with various ^{3}H-labeled sugars were mixed with [^{14}C]glucosamine-labeled desialated G-protein and chromatographed on Bio-Gel P6. As can be seen in Fig. 3, all three glycopeptide peaks were labeled with D-mannose, D-glucosamine, D-galactose, and L-fucose, indicating that the G-protein from rabies virus contains only oligosaccharide chains of the C_A type (6).

To achieve a greater resolution of the protease glycopeptides, the protease digestion

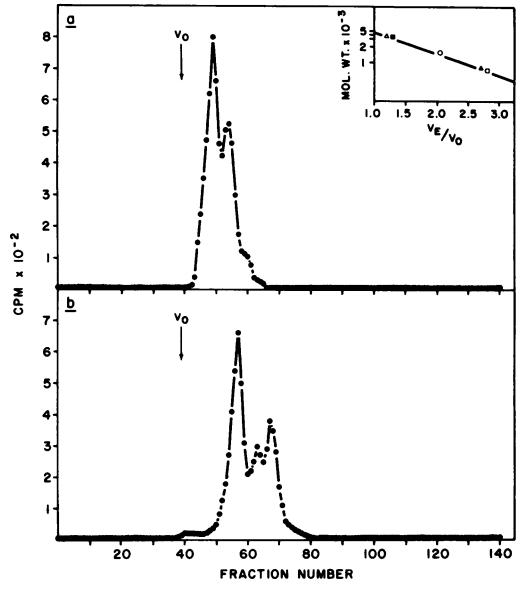

FIG. 2. *Bio-Gel P6 chromatography of glycopeptides from a protease digest of purified rabies virus glycoprotein before (a) and after (b) mild acid hydrolysis. Glucosamine-labeled G-protein was suspended in 0.1 M NH_4HCO_3 (pH 8.0) and exhaustively digested with protease. The digest was lyophilized. The lyophilisate was dissolved in a solution of 0.15 M Tris acetate (pH 7.8) and 0.1% SDS and chromatographed on a Bio-Gel P6 column. Blue Dextran 2000 was used as a marker for void volume. The molecular weights of the glycopeptides were estimated by comparison with the following markers on Bio-Gel P6: fetuin glycopeptide* (△), *thyroglobulin glycopeptide* (■), *ovalbumin glycopeptide* (○), *raffinose* (□), *and stachyose* (▲).

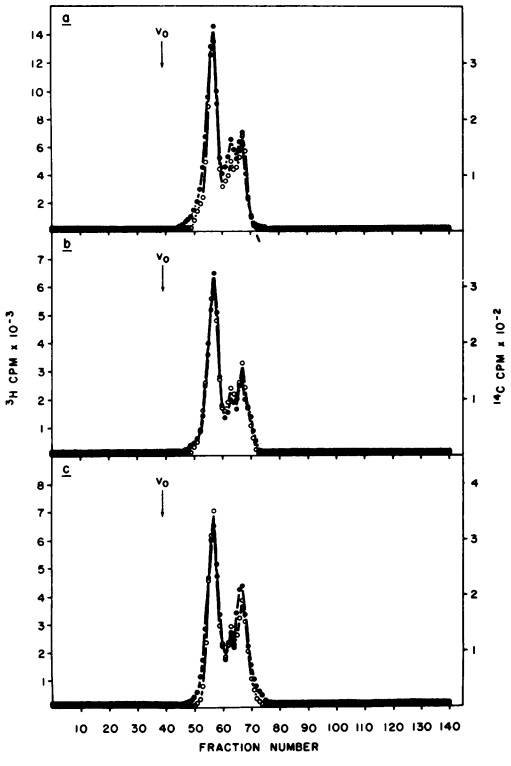

FIG. 3. *Gel filtration pattern of protease glycopeptides from desialated rabies virus G-protein labeled with various sugars. Glycopeptides from G-protein labeled with [^{3}H]fucose (a), [^{3}H]mannose (b), or [^{3}H]galactose (c) were chromatographed with a protease digest of [^{14}C]glucosamine-labeled G-protein on Bio-Gel P6.*

product was analyzed by DEAE-cellulose chromatography. Eight glycopeptides were resolved when the protease digestion product of [^{3}H]glucosamine-labeled rabies virus G-protein was chromatographed on DEAE-cellulose (Fig. 4a). To determine whether the multiple peaks found after DEAE-cellulose chromatography of protease-digested rabies G-protein reflected the heterogeneity in the sialic acid content of these glycopeptides, sialic acid residues were removed from the G-protein by mild acid hydrolysis. As shown in Fig. 4b, the number of peaks detected was reduced to three major and one or two minor glycopeptides when the G-protein was desialated before protease digestion, confirming the heterogeneity in sialic acid content.

The three major protease glycopeptides (A, B, and C) from desialated [^{3}H]glucosamine-labeled G-protein separated by DEAE-cellulose chromatography were then subjected to Bio-Gel P6 chromatography (Fig. 5a–c) to determine whether the desialated glycopeptides were homogeneous with respect to size. Each of the three glycopeptides co-chromatographed with a different one of the three peaks of the protease digestion product of [^{14}C]glucosamine-labeled desialated G-protein on Bio-Gel P6. The experiment also demonstrated that protease glycopeptide A (Fig. 4b), which did not bind to DEAE-cellulose, is a homogeneous species. The interrelationship of the three protease glycopeptides in Fig. 4b was examined by analysis of tryptic peptides of [^{3}H]glucosamine-labeled rabies virus G-protein on DEAE-cellulose: six separate tryptic glycopeptides were resolved by DEAE-cellulose chromatography (Fig. 6a). When the G-protein was desialated before trypsinization, only two major glycopeptides (I and III) and one minor glycopeptide (II) were detected (Fig. 6b). The same result was obtained when tryptic peptides produced from sialated G-protein were desialated by mild acid treatment to indicate that additional glycopeptides derived from sialated G-protein were not due to incomplete trypsin digestion (data not shown).

To determine the size of the individual desialated tryptic glycopeptides I, II, and III, obtained from desialated [^{3}H]glucosamine-labeled

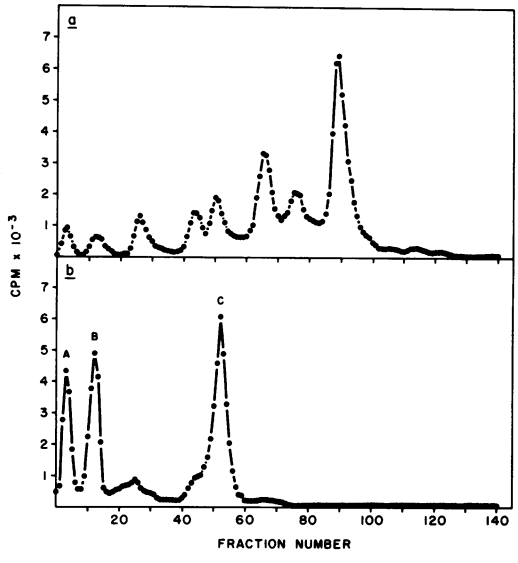

FIG. 4. *Analysis of glycopeptides of rabies virus G-protein obtained by protease digestion on a DEAE-cellulose column. (a) G-protein was purified from [^{3}H]glucosamine-labeled rabies virus and exhaustively digested with protease. The digest was then chromatographed on a DEAE-cellulose column as described in the text. (b) [^{3}H]glucosamine-labeled G-protein was hydrolyzed in 0.1 N H_2SO_4 at 80°C for 30 min. The hydrolysate was neutralized with Tris base, dialyzed against 0.1 M NH_4HCO_3 (pH 8.0), and digested with protease. After lyophilization, the protease peptides were analyzed on a DEAE column.*

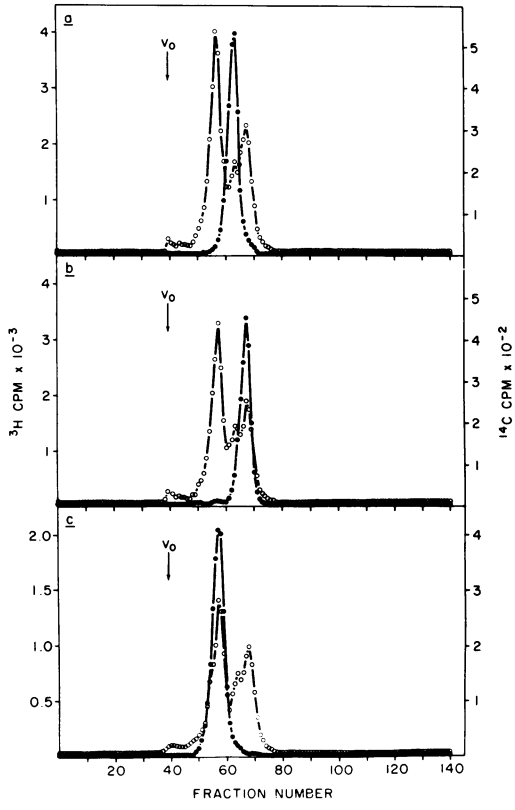

FIG. 5. *Chromatography of isolated protease glycopeptides on Bio-Gel P6. Desialated [^{3}H]glucosamine-labeled G-protein was digested with protease and the glycopeptides were separated by DEAE-cellulose chromatography. After dialysis and lyophilization, each of the three protease glycopeptides (A, B, and C as shown in Fig. 4b) was cochromatographed with a protease digest of [^{14}C]glucosamine-labeled desialated G-protein on Bio-Gel P6. (a) Protease glycopeptide A, (b) protease glycopeptide B, and (c) protease glycopeptide C.*

G-protein, each was separated by DEAE-cellulose chromatography and then subjected to Bio-Gel P6 chromatography (Fig. 7a–c). The results show that each of the three tryptic glycopeptides is homogeneous with respect to size. The following molecular weights were estimated: tryptic peptide I, 4,900; tryptic peptide II, 4,100; tryptic peptide III, 4,300.

The relationship between the desialated tryptic glycopeptides and desialated protease glycopeptides was determined by isolating the individual tryptic glycopeptides after DEAE-cellulose chromatography and extensively digesting with protease. The protein digest products were then rechromatographed on DEAE-cellulose (Fig. 8). Two [^{3}H]glucosamine-labeled glycopeptide peaks (A and B) were obtained when tryptic glycopeptide I (Fig. 6b) was digested with protease (Fig. 8a), and only one glycopeptide peak ("protease peptide" C) was obtained (Fig. 8c) after protease treatment of tryptic glycopeptide III (Fig. 6b). The elution position of protease peptide C is similar to that of the corresponding tryptic peptide III. Protease treatment of tryptic peptide II (Fig. 6b) produced predominantly protease peptide C and a small peak, which ran in the column in the position of protease peptide A (Fig. 8b). The amount of [^{3}H]glucosamine in this small peak was estimated to be only 3% of the total radioactivity corresponding to peak A, which was derived from tryptic peptide I.

Since tryptic glycopeptide I was resolved into two protease glycopeptides, the possibility that tryptic glycopeptide peak I consisted of two tryptic glycopeptides that were not resolved by the DEAE-cellulose chromatography was examined. Tryptic glycopeptide I was purified on DEAE-cellulose, dialyzed against 0.01 M NH_4HCO_3 (pH 8.0), lyophilized, and then dissolved in 0.2 N citric acid and rechromatographed on a Dowex AG 50 × 4 column. Figure 9 shows that this tryptic glycopeptide eluted from the cation exchanger as a single peak.

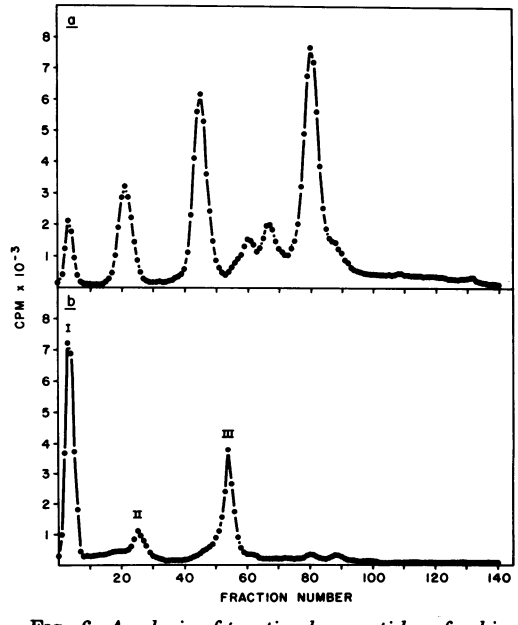

FIG. 6. *Analysis of tryptic glycopeptides of rabies virus G-protein on DEAE-cellulose. The G-protein was purified from [^{3}H]glucosamine-labeled rabies virus and digested with trypsin (a) or treated by mild acid hydrolysis and removal of sialic acid and neutralized and digested with trypsin (b). The tryptic peptides were analyzed on a DEAE-cellulose column as described in the text.*

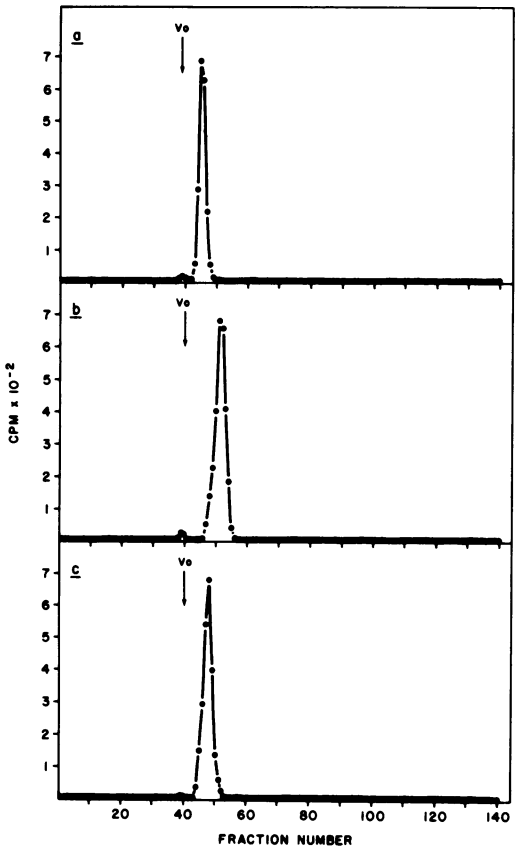

FIG. 7. *Chromatography of isolated tryptic glycopeptides on Bio-Gel P6. Desialated [³H]glucosamine-labeled G-protein was digested with trypsin, and the tryptic glycopeptides were separated by DEAE-cellulose chromatography. After dialysis and lyophilization, each of the three tryptic glycopeptides was chromatographed on Bio-Gel P6. (a) Tryptic glycopeptide I, (b) tryptic glycopeptide II, and (c) tryptic glycopeptide III.*

DISCUSSION

The purpose of these studies was to examine the structure of the rabies virus membrane G-protein and, in particular, to determine the number of oligosaccharide side chains. The results presented here allow us to conclude that there are three major oligosaccharides attached to the rabies virus G-protein.

Protease digestion of the rabies virus G-protein followed by chromatography on Bio-Gel P6 revealed two glycopeptides with molecular weights of 4,300 and 3,700. Removal of sialic acid resulted in the clear resolution of three glycopeptides by DEAE-cellulose chromatography, with molecular weights of 3,400, 2,800, and 2,500. Each of the three glycopeptides contained mannose, galactose, fucose, and glucosamine, identifying them as the so-called C_A-type glycopeptides. These three glycopeptides represent the major oligosaccharide units of rabies virus G-protein. Analysis of the protease or trypsin digestion products of rabies virus G-protein on DEAE-cellulose demonstrated eight protease glycopeptides or six tryptic peptides. However, when the G-protein was desialated before protease or trypsin digestion, the number of tryptic and protease glycopeptides was markedly reduced. The fact that trypsin or protease digestion produced more glycopeptides in untreated than in desialated rabies virus G-protein probably reflects the heterogeneity in the sialic acid content of the glycopeptides. By detailed analysis on DEAE-Sephadex of the tryptic digestion products of the G-protein from vesicular stomatitis virus, Robertson et al. (11)

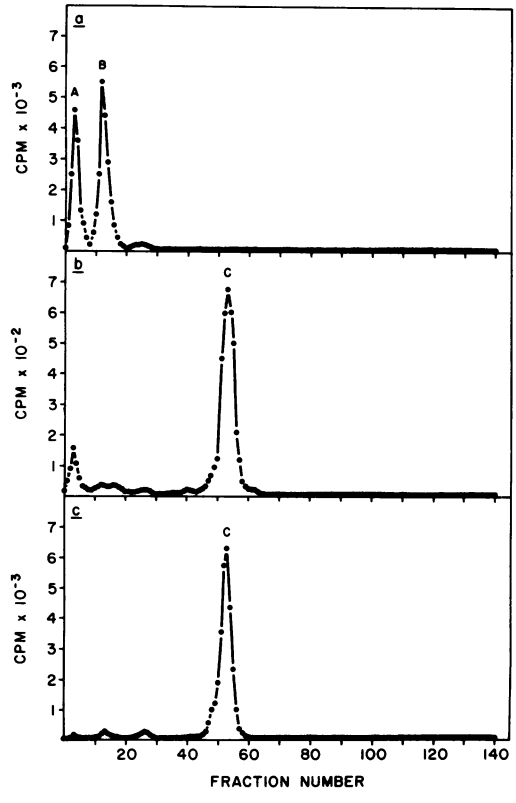

FIG. 8. *Analysis of glycopeptides obtained by protease treatment of isolated tryptic glycopeptides. Tryptic glycopeptides from desialated [³H]glucosamine-labeled rabies virus G-protein were separated by DEAE-cellulose chromatography and isolated, then dialyzed against 0.1 M NH_4HCO_3 (pH 8.0), and exhaustively digested with protease. The protease digestion products of tryptic peptides I (a), II (b), and III (c) were rechromatographed on DEAE-cellulose.*

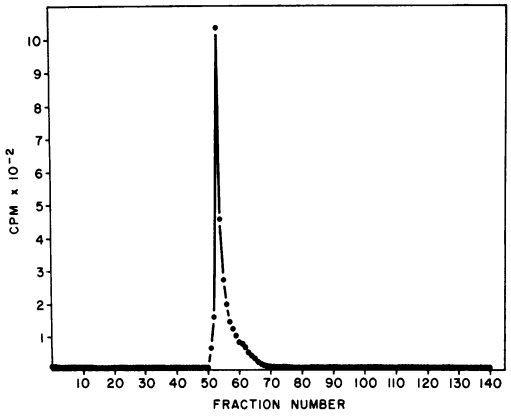

FIG. 9. *Analysis of tryptic peptide I on a Dowex AG 50 × 4 column. Tryptic peptide I of the tryptic peptides from desialated [³H]glucosamine-labeled G-protein that were separated on a DEAE-cellulose column was dialyzed against 0.01 M NH_4HCO_3 and lyophilized and dissolved in 0.2 N citric acid. The peptide was then chromatographed on a Dowex AG 50 × 4 column as described.*

demonstrated recently that vesicular stomatitis virus G-protein contains two oligosaccharide chains that are heterogeneous in their sialic acid content. The sialic acid content in the rabies virus G-protein is not constant but is affected by the growth conditions. G-protein from rabies virus grown at 37°C contains less sialic acid than that grown at 33°C. The effect of temperature on sialic acid content was also observed in Sindbis virus G-protein (7) and in Rous sarcoma virus G-protein (9).

The resolution of tryptic glycopeptide I as a single peak when analyzed on anionic and cationic exchangers as well as by Bio-Gel P6 chromatography suggests that this component was a single species. Although protease digestion of tryptic glycopeptide I produced two protease glycopeptides (A and B), it is, at present, uncertain as to whether tryptic peptide I contains one or two glycosylation sites. Since protease glycopeptides A and B have molecular weights of 2,800 and 2,500, respectively, one would predict that tryptic peptide I has a molecular weight of at least 5,300 if it contains two glycosylation sites. We estimated that tryptic glycopeptide I has a molecular weight of 4,900. Since the effect of conformational differences on the estimation of molecular weight is unknown in this system, it is our opinion that the close approximation of the measured and predicted molecular weights of tryptic peptide I suggests it has two sites of glycosylation. However, in the absence of a detailed analysis of amino acids and sugars associated with protease peptides A and B, we cannot exclude the possibility that protease peptides A and B originated from the same glycosylation site and differ only by the ragged peptide remaining. However, the observation that protease peptides A and B appear to be homogeneous and discrete with respect to size and elution from DEAE-cellulose and the fact that protease peptides A and B were reproducibly detected repeatedly in nearly the same ratio argues against the latter possibility. Protease treatment of tryptic glycopeptide III only produced one peptide (protease peptide C). Furthermore, protease digestion of the minor tryptic glycopeptide II also produced mainly protease peptide C. At present, it is unclear whether tryptic glycopeptide II represents a distinct glycosylation site that is glycosylated only in a small number of molecules or whether it is a degradation product of tryptic glycopeptide III.

Schlumberger et al. (12) determined that 2.9% of the total protein mass in rabies virus are protein-bound carbohydrates. Since the G-protein accounts for 25% of the rabies virus proteins (14), we have calculated that on the average, each molecule of G-protein contains 9,280 daltons of carbohydrate including sialic acid. If we assume that protease glycopeptides A, B, and C represent distinct sites of glycosylation and exhibit an approximate one-to-one stoichiometry, one can hypothesize that each desialated G-protein molecule contains three carbohydrate side chains. According to this hypothesis, the total molecular weight of these side chains would be 7,830 after correction for the approximate amino acid (approximately 10%) content of these desialated peptides (3). Since the data presented in this paper indicated that the majority of the carbohydrate side chains contain sialic acid, we can assume that each side chain contains a minimum of one sialic acid residue. On this assumption, the total molecular weight of the three sialated side chains would be 8,760. This value closely approximates the value of 9,280 daltons obtained by direct carbohydrate analysis and is consistent with the argument that the majority of the G-protein molecules contain three oligosaccharide side chains.

ACKNOWLEDGMENTS

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