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Characterization of Oligosaccharide Structures on a Chimeric Respiratory Syncytial Virus Protein Expressed in Insect Cell Line Sf9

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ABSTRACT: The oligosaccharide structures added to a chimeric protein (FG) composed of the extracellular domains of respiratory syncytial virus F and G proteins, expressed in the insect cell line Sf9, were investigated. Cells were labeled in vivo with [^{3}H]glucosamine and infected with a recombinant baculovirus containing the FG gene. The secreted chimeric protein was isolated by immunoprecipitation and subjected to oligosaccharide analysis. The FG protein contains two types of O-linked oligosaccharides: GalNAc and Galβ1-3GalNAc constituting 17 and 66% of the total number of structures, respectively. Only one type of N-linked oligosaccharide, constituting the remaining 17% of the structures on FG, was detected: a trimannosyl core structure with a fucose residue linked α1-6 to the asparagine-linked *N*-acetylglucosamine.

The human respiratory syncytial virus (RSV)[1] genome codes for 10 proteins. Two of these, the F and G proteins, are expressed at the surface of the infected cell and the viral envelope and are important for cell fusion and viral attachment, respectively (Gruber & Levine, 1983; Levine et al., 1987). Both proteins are anchored to the membrane by hydrophobic domains that have been localized to the C-terminal end of the F protein (Collins et al., 1984) and the N-terminal end of the G protein (Wertz et al., 1985).

The F and G glycoproteins have been shown to be the principal targets of the host antibody response to RSV (Murphy et al., 1986). Vaccination with either of these glycoproteins will induce neutralizing antibodies and will protect animals from RSV challenge (Olmstead et al., 1986; Walsh et al., 1987). Since the F and G glycoproteins serve different viral functions (cell fusion and viral attachment, respectively), we reasoned that a vaccine inducing an immune response against both glycoproteins should be optimal. A chimeric, secreted protein (FG) composed of the signal and extracellular domains of the F protein linked to the extracellular domain of the G protein was constructed and expressed in the insect cell line Sf9 by using a baculovirus vector (Wathen et al., 1989). Animals vaccinated with this chimeric protein elicited an antibody response against both the F and G portions of the chimeric protein and were protected against RSV challenge (Brideau et al., 1989a). Generating a chimeric protein has the practical advantage of being able to produce and purify a single protein for a vaccine against RSV.

When synthesized by a mammalian cell, the F protein is a 68-kDa glycoprotein containing approximately 9 kDa N-linked oligosaccharides; no evidence for O-linked glycosylation has been found (Lambert, 1988). The mature G protein has a molecular mass of 90 kDa in mammalian cells and is heavily glycosylated. Approximately 12 kDa of the molecular mass on SDS–PAGE is contributed by N-linked oligosaccharides and as much as 45 kDa by O-linked oligosaccharides (Lambert, 1988; Wertz et al., 1989). The extensive O-glycosylation on the G protein is believed to be an important factor in the human immunoresponse to RSV (Wagner et al., 1989).

Several authors have reported that oligosaccharide structures synthesized by insect cells differ from those synthesized by mammalian cells [e.g., Butters and Hughes (1981), Hsieh and Robbins (1984), Wojchowski et al. (1987), Greenfield et al. (1988), Weber et al. (1986) and Kuroda et al. (1990)]. It has been demonstrated that *Aedes albopictus* cells do not synthesize complex-type oligosaccharides but they instead substitute truncated high-mannose structures on glycosylation sites that would contain complex-type structures if the protein was synthesized by a mammalian cell. Other authors have shown that insect cells appear to lack several of the glycosyltransferases involved in the addition of terminal sugars to N-linked oligosaccharides (Butters et al., 1981). O-Linked glycosylation also appears to differ, at least in Sf9 cells; thus, it has been demonstrated that these cells only to a limited extent substitute O-linked *N*-acetylgalactosamine with galactose and that, in analogy with N-linked oligosaccharides, no terminal sialic acid is added (Thomsen et al., 1990).

In this report we present a characterization of the oligosaccharide structures added to the FG protein when synthesized by the insect cell line Sf9.

Materials and Methods

D-[6-^{3}H]Glucosamine hydrochloride (32 Ci/mmol), D-[1-^{14}C]glucosamine hydrochloride (54 mCi/mmol), and sodium boro[^{3}H]hydride (407 mCi/mmol) were purchased from Amersham Corp. EN^{3}HANCE was from New England Nu-

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[1] Abbreviations: GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; Gal, galactose; Man, mannose; Fuc, fucose; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; AcNPV, *Autographa californica* nuclear polyhedrosis virus; Sf9, *Spodoptera frugiperda* cell line 9; RSV, respiratory syncytial virus; MOI, multiplicity of infection; PFU, plaque-forming units; Con A, concanavalin A.

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clear. 2-Acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactopyranose (Galβ1-3GalNAc), *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose, glucose, mannose, fucose, rhamnose, α-methyl mannoside, α-methyl glucoside, Amberlite MB-3, Dowex 50W-X8, jack bean β-galactosidase, jack bean α-mannosidase, bovine epididymis α-fucosidase, and endoglycosidase D were from Sigma. Bovine testis β-galactosidase and endo-α-*N*-acetylgalactosaminidase were from Boehringer. Pronase was from Calbiochem. Protein A–Sepharose and concanavalin A–Sepharose were obtained from Pharmacia. Pea lectin–agarose was from Vector Laboratories. Rabbit anti-mouse IgG was purchased from Accurate Chemical Corp. Grace's insect medium, fetal bovine serum, penicillin, streptomycin, and fungizone were purchased from Gibco. Isolation of the anti-FG monoclonal antibodies (Mab 1-112 and Mab 2-27) has been described (Brideau et al., 1989b).

The radiolabeled Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc and Manβ1-4GlcNAcβ1-4GlcNac standards were prepared by exoglycosidase digestion of an in vivo [^{3}H]mannose-labeled biantennary oligosaccharide isolated from a recombinant tissue plasminogen activator analogue (Aeed and Elhammer, unpublished results). All other reagents were from standard sources.

Baculovirus Constructions. Construction of the RSV FG gene and its expression in a baculovurus vector have been described previously (Wathen et al., 1989). Briefly, a chimeric gene was constructed in which the DNA sequences coding for the signal and extracellular regions of the RSV F glycoprotein were ligated to the DNA sequences coding for the extracellular region of the RSV G glycoprotein. The chimeric FG gene was placed under control of the baculovirus polyhedrin promoter by cloning into the *Bam*HI site of the baculovirus transfer vector pAc 373. Transfer of the FG gene into the baculovirus genome was accomplished by cotransfection of the transfer plasmid and wild-type baculovirus DNA into Sf9 cells according to a calcium phosphate precipitation procedure. Recombinant virus containing the FG gene and displaying the characteristic occlusion-negative plaque morphology was plaque-purified twice.

Cell Culture and Preparation of Radiolabeled FG Protein. The baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) was grown in Sf9 cell cultures (ATCC, CRL 1711). Conditions for growth of cells and virus have been described (Summers & Smith, 1987). Sf9 cells were infected with recombinant baculovirus containing the FG gene at a MOI of 10 PFU/cell. Twenty-four hours postinfection the cells were washed twice with PBS and suspended in glucose-free Grace's insect medium containing 10% fetal bovine serum and 10 μCi/mL [^{14}C]glucosamine or 100 μCi/mL [^{3}H]-glucosamine (see individual experiments). At 48 h postinfection the medium was removed and clarified by low-speed centrifugation. The FG protein was immunoprecipitated from the medium with use of RSV-specific monoclonal antibodies, as described previously (Wathen & Wathen, 1984). The protein A–Sepharose used to precipitate the antigen–antibody complexes was precoated with rabbit anti-mouse IgG. The purity of the immunoprecipitated FG protein was investigated on SDS–PAGE followed by fluorography. Only one radioactive band migrating at approximately 105 kDa was detected (data not shown). For oligosaccharide analysis, either the intact FG protein was released from the protein A–Sepharose–antibody complex by boiling in 2% SDS and the detergent was subsequently removed from the SDS-solubilized protein as described by Wessel and Flugge (1984) or, alternatively, following SDS–PAGE, radiolabeled glycopeptides were released by Pronase digestion of the antigen band (Cummings et al., 1983).

Oligosaccharide Analysis. For analysis of the O-linked oligosaccharides on the FG protein, an aliquot from the intact radiolabeled protein fraction or from the Con A run-through fraction, pool I (see below), was subjected to alkaline sodium borohydride treatment essentially as outlined by Carlson (1968). The released oligosaccharides were separated on descending paper chromatography in pyridine–ethyl acetate–glacial acetic acid–H_2O (5:5:1:3). Galβ1-3GalNAc was released in unreduced form by digestion with endo-α-*N*-acetylgalactosaminidase (0.5 unit/mL) in 20 mM Tris–maleate, pH 6.0, and 10 mM galactonolactone at 37 °C for 24 h. The released disaccharide was separated from undigested radioactivity on a Bio-Gel P-2 column (1.0 × 45 cm) equilibrated in 7% 2-propanol. Thirty 1.6-mL fractions were collected. V_0 was determined by using bovine serum albumin. Digestion with bovine testis and jack bean β-galactosidase was carried out as described by Cummings et al. (1989).

N-Linked oligosaccharides were isolated on concanavalin A–Sepharose essentially as described by Cummings et al. (1983); only one eluant was used, 100 mM α-methyl mannoside at 55 °C. Conditions for strong acid hydrolysis and separation of labeled amino sugars is described by Cummings et al. (1989). Pea lectin chromatography was carried out as described by Cummings and Kornfeld (1982) with minor modifications; again, only one eluant was used, 500 mM α-methyl mannoside. Hydrazinolysis was done as described by Takasaki et al. (1982). Analytical size-exclusion chromatography was performed on a calibrated Bio-Gel P-4 column (1.5 × 200 cm) as outlined by Yamashita et al. (1982); 0.9-mL fractions were collected. Digestion with bovine epididymis α-fucosidase (0.16 unit/mL) was done in 30 mM citrate, pH 4.5, at 37 °C for 60 h; jack bean α-mannosidase (14 units/mL) digestion was carried out in 50 mM sodium acetate, pH 5.0, at 37 °C for 48 h; endoglycosidase D (0.3 unit/mL) digestion was in 100 mM citrate–phosphate, pH 6.5, at 37 °C for 40 h.

In order to determine which carbon atom on the *N*-acetylglucosamine in the reducing end of the N-linked oligosaccharide is substituted with fucose, a periodate oxidation experiment was carried out as described by Yamashita et al. (1979), with some modifications. Briefly, recombinant baculovirus infected cells were labeled in vivo with [^{14}C]-glucosamine. Labeled FG protein was isolated by immunoprecipitation as described above and released from the immunomatrix by boiling in 2% SDS. Following removal of the detergent (see above), the protein precipitate was dried extensively under vacuum, and the radiolabeled oligosaccharides were released by hydrazinolysis as described above. The oligosaccharides were isolated, identified, and digested with endoglycosidase D as described above. The Fuc–GlcNAc fragment was solubilized in 50 mM NaOH and reduced with $NaBH_4$ essentially as described by Takasaki et al. (1982). The following periodate oxidation, reduction, mild acid hydrolysis, and separation of the resulting fragments were carried out as described by Yamashita et al. (1979), as was the preparation of the chromatography standards used.

Monosaccharide composition was determined with use of a Dionex liquid chromatograph equipped with a CarboPac PA1 column and pulsed amperometric detection (Hardy et al., 1988). Flow rate was 1 mL/min.

Results

Pronase digestion of the intact in vivo radiolabeled FG protein followed by separation of the resulting glycopeptides

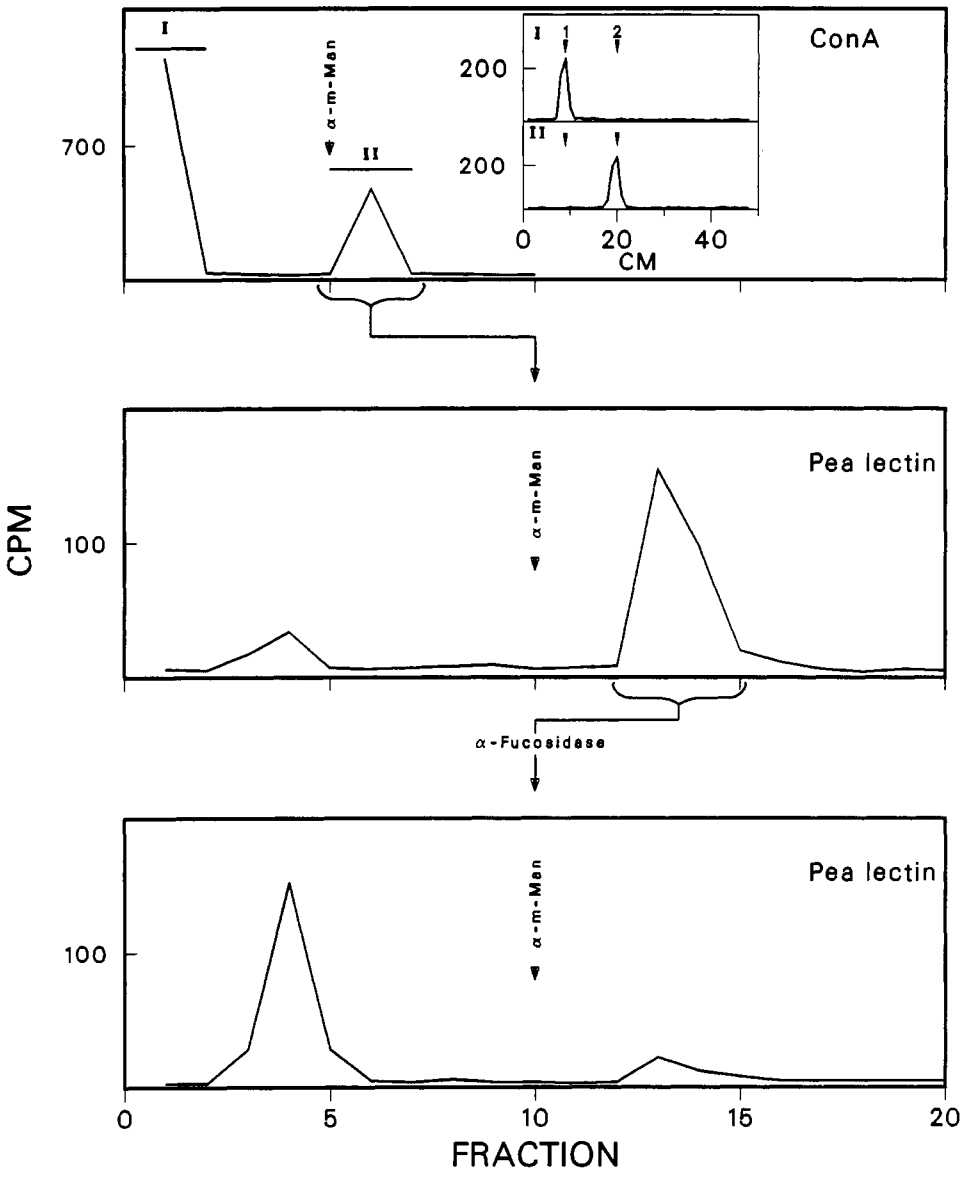


FIGURE 1: Lectin chromatography of FG glycopeptides. Cells were infected with a recombinant baculovirus containing the FG gene, labeled for 24 h (24–48 h postinfection) with [^{3}H]glucosamine, and the secreted FG protein was isolated by immunoprecipitation as described under Materials and Methods. The radiolabeled FG protein was subsequently digested with Pronase, and the resulting glycopeptides were fractionated on Con A–Sepharose (top panel). The run-through and bound fractions were analyzed for amino sugar content (top panel, inset) and pooled as indicated. Pool II was further analyzed on pea lectin agarose before and after digestion with bovine epididymis α-fucosidase (middle and bottom panels, respectively). Bound material was eluted with 500 mM α-methyl mannoside. The migration of standards is indicated: (1) GalNAc; (2) GlcNAc.

on concanavalin A–Sepharose resulted in the profile shown in Figure 1, top panel. Approximately 39% of the radioactivity bound to and could specifically be eluted from the column. Strong acid hydrolysis of the radioactive material in the two Con A fractions, followed by separation of the released labeled amino sugars on paper chromatography, showed that all the radioactivity in the run-through fractions was present in *N*-acetylgalactosamine while in the Con A binding fractions all the radioactivity was in *N*-acetylglucosamine (Figure 1, top panel, inset); thus, all N-linked oligosaccharides on the FG protein appear to bind to Con A. In addition, >97% of the radioactivity in the Con A run-through fraction could be released with alkaline sodium borohydride (see below) while the structures in the Con A binding fraction were essentially resistant to this treatment (data not shown).

Separation of the oligosaccharide structures released by mild alkaline sodium borohydride treatment of either intact FG protein or glycopeptides in Con A fraction I (see above) on descending paper chromatography resulted in the profile shown in Figure 2. Control experiments showed that >97% of the protein-bound GalNAc was released by the conditions used (data not shown). Twenty-one percent (for the intact protein, 22%; data not shown) of the radioactivity migrated in the position of GalNac-ol; the remaining 78% migrated in the position of Galβ1-3GalNAc-ol. In order to identify this second structure, glycopeptides from the Con A run-through fraction were digested with endo-α-*N*-acetylgalactosaminidase, and the released structures were separated by Bio-Gel P-2 chromatography. Seventy-nine percent of the radioactivity applied to the column was recovered in the fractions containing the released structures, consistent with essentially all disaccharides on FG being sensitive to the enzyme (data not shown). Endo-α-*N*-acetylgalactosaminidase specifically cleaves the linkage between GalNAc in the structure Galβ1-3GalNAc and Ser/Thr on the protein (Endo & Kobata, 1976; Umemoto et al., 1977). Subsequent digestion of the released oligosaccharides with jack bean and bovine testis β-galactosidases showed that the putative disaccharide contains a terminal

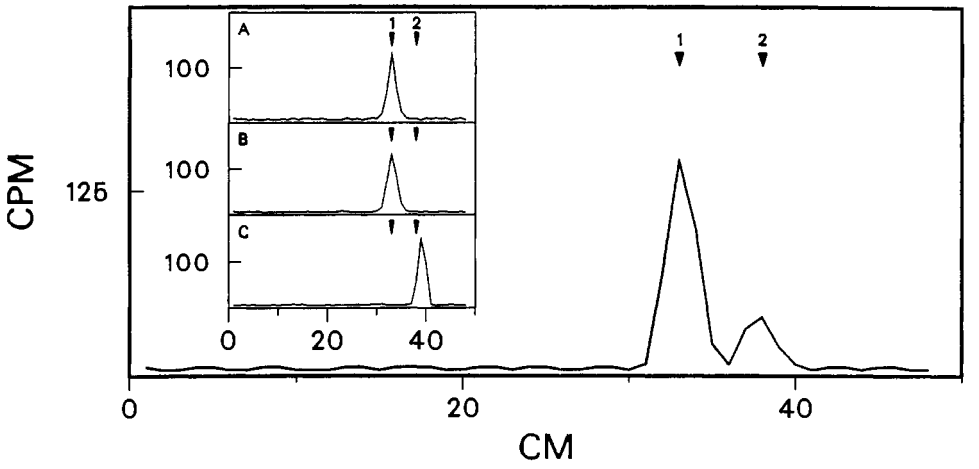


FIGURE 2: Paper chromatography of FG oligosaccharides released by mild alkaline sodium borohydride treatment. An aliquot of the glycopeptides in pool I from Con A–Sepharose chromatography (see Figure 1) was subjected to mild alkaline sodium borohydride treatment, and the released oligosaccharides were separated on descending paper chromatography in pyridine–ethyl acetate–glacial acetic acid–H_2O (5:5:1:3). The putative disaccharide was further analyzed by exoglycosidase treatment (inset). FG oligosaccharides released by endo-α-*N*-acetylgalactosaminidase digestion were digested extensively with β-galactosidases as described under Materials and Methods, and the resulting products were separated on descending paper chromatography in pyridine–ethyl acetate–glacial acetic acid–H_2O (5:5:1:3). Key: (A) undigested oligosaccharide; (B) products from digestion with jack bean β-galactosidase; (C) products from digestion with bovine testis β-galactosidase. The migration of standards is indicated: (1) Galβ1-3GalNAc-ol; (2) GalNAc-ol.

β1-3-linked galactose residue linked to *N*-acetylgalactosamine (Figure 2, inset). Bovine testis β-galactosidase readily cleaves β1-3 and β1-4 linkages but cleaves β1-6 linkages only slowly; jack bean β-galactosidase cleaves β1-4 and β1-6 linkages but not β1-3 linkages under the conditions used (Uchida et al., 1979; Li et al., 1975).

Further lectin fractionation of the glycopeptides binding to Con A showed that the majority of the radioactivity in this fraction bound to and could be specifically eluted from pea lectin (Figure 1, middle panel); digestion of the material with bovine epididymis α-fucosidase almost completely abolished this interaction (Figure 1, bottom panel). The oligosaccharides in the Con A binding fractions were released by hydrazinolysis and fractionated on a calibrated Bio-Gel P-4 column. Only one radioactive peak was detected, eluting at the position of an oligoscaccharide containing 8 glucose units (Figure 3A). Eight glucose units is the elution position of a core-fucosylated biantennary structure after extensive digestion with β-galactosidase and β-hexosaminidase, i.e., a trimannosyl structure with a fucose residue linked to the innermost *N*-acetylglucosamine (see Figure 3, standard I). Digestion of the structure or structures in the 8-glucose-units peak with bovine epididymis α-fucosidase resulted in a 1-glucose-unit shift in elution position, consistent with the removal of 1 terminal fucose residue (Figure 3B). Further digestion with jack bean α-mannosidase caused the radioactive peak to shift an additional 1.6 glucose units, consistent with the removal of 2 outer mannoses (Figure 3C). Digestion of the intact oligosaccharide with endoglycosidase D resulted in two peaks, containing equal amounts of radioactivity, eluting at the positions of oligosaccharides containing 4.7 and 3.3 glucose units, respectively (Figure 3D); *N*-acetylglucosamine elutes at 2 glucose units in this system. Endoglycosidase D cleaves N-linked structures with unsubstituted α1-3-linked core mannose between the two core *N*-acetylglucosamines (Muramatsu, 1978). Taken together, these experiments suggest that hydrazinolysis releases only one type of oligosaccharide from the FG protein: a trimannosyl structure with a fucose linked to the innermost *N*-acetylglucosamine.

The very limited amounts of FG protein available made it impractical to perform a methylation analysis on the oligosaccharide structures. Thus, in order to determine the location

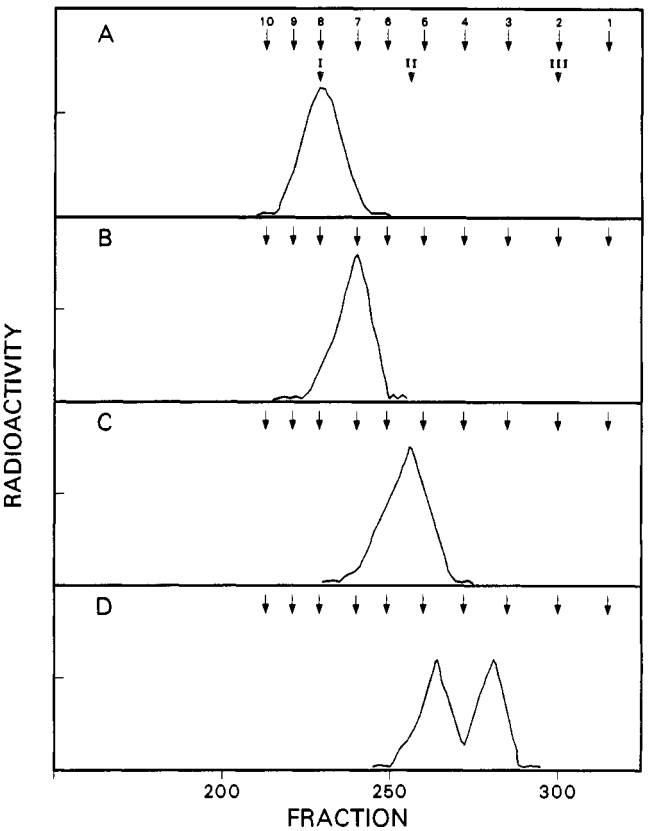
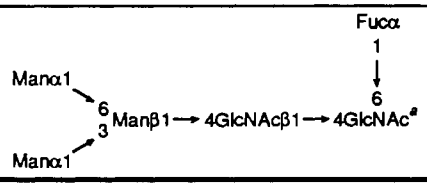


FIGURE 3: Sequential glycosidase digestion of FG oligosaccharides released by hydrazinolysis. The glycopeptides in Con A–Sepharose chromatography pool II were subjected to hydrazinolysis, and the released oligosaccharides were fractionated on a calibrated Bio-Gel P-4 column (1.5 × 200 cm). Key: (A) intact hydrazinolysis-released oligosaccharide; (B) product from digestion with bovine epididymis α-fucosidase; (C) product from digestion with bovine epididymis α-fucosidase and jack bean α-mannosidase; (D) product from digestion with endoglycosidase D. Numbers 1–10 indicate the elution volumes of dextran oligomer standards composed of 1–10 glucose units. Labels: (I) Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc; (II) Manβ1-4GlcNAcβ1-4GlcNAc; (III) GlcNAc.

of the fucosyl residue on the reducing GlcNAc, the 3.3-glucose-units fragment from a preparation of in vivo [1-^{14}C]glucosamine-labeled FG oligosaccharides was isolated,

Table I: Summary of the Oligosaccharide Structures Detected on the FG Protein

structure	relative amount (%)
GalNAcOH	17
Galβ1→3GalNAcOH	66
	17

[a] Linkage positions proposed in accordance with the common structures on N-linked oligosaccharides and previously published data on glycosylation in insect cells (Kornfeld & Kornfeld, 1985; Hsieh & Robbins, 1984).

Table II: Monosaccharide Composition of the FG Protein[a]

	amount (mol/mol of protein)[b]	molar ratio[c]	molar ratio[c] predicted by oligosaccharide analysis[d]
fucose	7.1	1.3	1.0
mannose	16.7	3.0	3.0
galactose	22.7	4.1	3.9
N-acetylgalactosamine	27.6	4.9	4.9
N-acetylglucosamine	9.5	1.7	2.0

[a] The monosaccharide composition of purified FG protein was determined on a Dionex chromatograph as outlined under Materials and Methods. [b] Protein was determined by amino acid analysis. [c] Ratios were calculated by setting the number of mannose residues to 3.0. [d] The ratio predicted by oligosaccharide analysis was calculated from the distribution of FG oligosaccharides shown in Table I.

reduced, and subjected to periodate oxidation as described under Materials and Methods. Paper-chromatographic separation of the fragments formed by mild acid hydrolysis of the oxidized material resulted in only one peak, migrating in the position of 2-acetamidopropanediol (data not shown). Theoretically, this should be the only radioactive fragment formed from [1-^{14}C]glucosaminitol when substituted on carbon atom 6. The sensitivity of the oligosaccharide to bovine epididymis α-fucosidase (see above) strongly suggests that the oligosaccharide contains a terminal α-linked fucose residue. Taken together, these experiments indicate that the fucose is linked α1-6 to the asparagine-linked *N*-acetylglucosamine.

Table I summarizes the oligosaccharide structures detected on the chimeric FG molecule.

The total monosaccharide composition of the FG protein is shown in Table II. The ratios between the different monosaccharide constituents compare quite well to the predicted ratios from the oligosaccharide analysis.

Discussion

The current investigation clearly demonstrates that the FG molecule, in analogy with its component proteins, contains a number of oligosaccharide structures, both N-linked and O-linked. The monosaccharide composition (Table II) is consistent with 5–6 N-linked structures (the chimeric molecule contains 8 potential sites) and 24–25 O-linked structures per molecule. The different types of structures are summarized in Table I. Only one type of N-linked oligosaccharide was detected, a fucosylated trimannosyl core. Previous investigations have established that insect cells appear not to add the outer sugars commonly found in processed N-linked oligosaccharides synthesized by mammalian cells (Butters & Hughes, 1981; Butters et al., 1981; Hsieh & Robbins, 1984; Domingo & Throwbridge, 1988; Greenfield et al., 1988; Weber et al., 1986; Kuroda et al., 1990). Rather, they synthesize the high-mannose precursor, utilizing an assembly system similar to that of mammalian cells, and may subsequently trim this structure to varying degrees by removal of 1–6 of the outer mannoses. Thus, mature secreted proteins contain only intact or truncated high-mannose-type oligosaccharides. However, an exception to this rule has recently been published (Davidson et al., 1990). The mature F and G proteins synthesized by mammalian cells appear to contain all complex type (endoglycosidase H resistant) N-linked structures (Lambert, 1988; Wertz et al., 1989). The presence of only truncated N-linked structures on the FG protein, therefore, agrees with the observation made on oligosaccharides synthesized by *A. albopictus* cells, that this type of structure is most frequently found on sites substituted with complex-type structures by mammalian cells (Hsieh & Robbins, 1984). The fucose substitution of the asparagine-linked *N*-acetylglucosamine is consistent with reported data on monosaccharide composition (Butters & Hughes, 1981; Gorman & Hosick, 1982) and reactivity with fucose-specific lectins (Russel & Consigli, 1985; Cox & Willis, 1987) of glycoproteins synthesized by insect cells. Furthermore, similar structures have previously been tentatively identified on bee venom phospholipase A_2 and more recently on influenza hemagglutinin synthesized by Sf9 cells (Weber et al., 1986; Kuroda et al., 1990). In neither of these cases, however, was the substituted carbon atom on the asparagine-linked *N*-acetylglucosamine determined. It is of interest to note that mammalian cells reportedly are incapable of synthesizing this type of structure; the mammalian fucosyltransferase has an absolute requirement for the prior attachment of an *N*-acetylglucosamine residue to the Man_3-$GlcNAc_2$ core (Wilson et al., 1976).

The chimeric FG molecule contains two types of O-linked oligosaccharides, together constituting approximately 83% of the total number of structures. In agreement with previous work on O-linked glycosylation in Sf9 cells, none of the structures contain sialic acid, and the largest core is the disaccharide Galβ1-3GalNAc (Thomsen et al., 1990). The considerable number of structures on the molecule is consistent with the apparently extensive substitution with O-linked structures found on the G protein expressed in mammalian cells (Wertz et al., 1989).

The G protein of RSV induces a weaker immune response in animal models than the F glycoprotein (Olmstead et al., 1986), and analysis of the serum IgG antibody subclass response in humans has indicated that the G glycoprotein induces an abnormally low IgG1/IgG2 ratio (Wagner et al., 1989). It has been hypothesized that this unusual immune response is due to the extensive glycosylation of the G protein in mammalian cells. The chimeric FG glycoprotein appears to be more heavily glycosylated in mammalian cells than in insect cells. Analysis on SDS–polyacrylamide gels has shown that the molecular size of FG produced in mammalian cells is approximately 138 kDa, compared to 105 kDa in insect cells (data not shown). A large portion of this difference is probably caused by the presence of generally smaller truncated structures and the absence of sialic acid on the insect-produced protein. It should be interesting to determine if FG synthesized by the two different cell types will induce different immune responses in animals.

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