Processing of the Asparagine-linked Oligosaccharides of Secreted and Intracellular Forms of the Vesicular Stomatitis Virus G Protein: In Vivo Evidence of Golgi Apparatus Compartmentalization

CHRISTOPHER A. GABEL and JOHN E. BERGMANN
Department of Anatomy and Cell Biology, Columbia University College of Physicians and Surgeons, New York, New York 10032

ABSTRACT The structures of the asparagine-linked oligosaccharides of several variant forms of the vesicular stomatitis virus glycoprotein transiently expressed from cloned cDNAs have been determined. Glycopeptides isolated from forms of the G protein that reach the cell surface or that are secreted into the medium are virtually identical; they contain complex-type oligosaccharides whose nonreducing ends terminate in galactose and sialic acid residues. In contrast, forms of the G protein that remain intracellular possess oligosaccharides at intermediate stages in the processing pathway. One deletion mutant, Δ1473, codes for a protein that remains in the rough endoplasmic reticulum (Rose, J. K., and J. E. Bergmann, 1982, *Cell*, 30:753–762) and contains only high mannose–type oligosaccharides. Another mutant, Δ1554, codes for a glycoprotein that contains oligosaccharides of primarily two classes. One class is of the high mannose type and is similar to those found on the protein coded for by Δ1473. However, the major class contains biantennary and more highly branched complex-type oligosaccharides that terminate in *N*-acetylglucosamine rather than galactose or sialic acid residues. These data suggest that the protein coded for by Δ1554 migrates to the Golgi apparatus, but does not enter the more distal compartment(s) of the organelle which contains galactosyl- and sialyltransferases.

The biosynthesis of complex-type asparagine-linked oligosaccharides is a highly ordered process. It begins with the transfer of a common oligosaccharide precursor from dolichol pyrophosphate to the nascent polypeptide (1). After the completion of protein synthesis, the protein-bound oligosaccharides are modified through the combined action of specific glycosidases and glycosyltransferases. While many of the steps in the biosynthetic pathway have been identified (2–4), little is known concerning the regulation of the individual processing enzymes or of the factors that determine the final structure of a glycoprotein's oligosaccharides.

There are many potential mechanisms that may be operating to affect processing of oligosaccharides on glycoproteins. For example, a recent review by Pollack and Atkinson (5) noted a correlation between the position of an asparagine-linked oligosaccharide relative to the protein's amino terminus and the likelihood of the oligosaccharide becoming a complex-type structure. It was also noted that the asparagine-linked oligosaccharides on secretory proteins are more likely to be complex-type structures than are oligosaccharides on membrane-bound glycoproteins. These observations suggest that the positioning of an oligosaccharide relative to the membrane may restrict its accessibility to the processing enzymes. A second possible mechanism is that the oligosaccharide processing enzymes may be sequestered into different compartments, such that a newly synthesized glycoprotein would be exposed to only one of several enzymes capable of modifying it. There is strong evidence in favor of this type of enzyme compartmentalization. Not only is there a clear distinction between enzymes found in the endoplasmic reticulum and those found in the Golgi apparatus (6), but there is increasing evidence that the Golgi-associated late-stage oligosaccharide processing enzymes are arranged non-uniformly. For example, morphological studies have shown that galac-

THE JOURNAL OF CELL BIOLOGY · VOLUME 101 AUGUST 1985 460-469

tosyltransferase is localized to the *trans*-most cisternae of the Golgi complex in HeLa cells (7). Moreover, biochemical analyses of Golgi membranes fractionated on sucrose density gradients have indicated that many of the oligosaccharide processing enzymes sediment at different densities, which suggests that the enzymes are localized to separate membranes (8–10).

The prediction from these observations is that the Golgi apparatus is arranged so that a newly synthesized glycoprotein present at the *cis* face of the organelle is not accessible to the processing enzymes restricted to the *trans* face. As such, if a protein that contains asparagine-linked oligosaccharides were to remain within the *cis* Golgi region, the structure of the oligosaccharide units should be incomplete, possessing only those modifications performed by enzymes localized within this compartment. Although many researchers have succeeded in isolating early processing intermediates (glycoproteins modified by the glycosidases of the endoplasmic reticulum), no late stage oligosaccharide processing intermediates have been isolated. This is presumably due to the fact that processing proceeds rapidly once the newly synthesized glycoproteins reach the Golgi apparatus. Asparagine-linked oligosaccharide processing intermediates have been isolated from lectin-resistant cell mutants that are blocked at various points along the processing pathway (11).

For this study, we used recombinant DNA technology to evaluate the effect of several factors on processing of a glycoprotein's asparagine-linked oligosaccharides. Rose and Bergmann (12, 13) previously isolated and characterized a family of cDNAs coding for glycoproteins related to the G protein of vesicular stomatitis virus (VSV).[1] These cDNAs, coupled to the late SV40 promoter, were expressed transiently in COS-1 cells. The expressed proteins share with the G protein a common amino terminal domain (90% of the molecule), which contains both its asparagine-linked oligosaccharides. They differ from the G protein in lacking all, or part of, its carboxy terminal transmembrane and cytoplasmic domains. Some of these proteins are secreted, some reach the plasma membrane where they remain membrane bound, and others are restricted to intracellular sites. As such, the truncated G proteins offer a unique opportunity to investigate the degree to which a glycoprotein's membrane association and intracellular migration affect processing of its asparagine-linked oligosaccharides.

MATERIALS AND METHODS

Cell Transfection: The plasmid DNAs were the same as those described by Rose and Bergmann (12, 13); the deletion mutants were orginally referred to by the number of the nucleotide in the G protein mRNA to which the deletion extends. For the sake of brevity, we have used the convention of naming the truncated protein specified by a deletion mutant after the mutant (i.e., del-1554 is the protein specified by Δ1554).

The procedure for DNA transfection of COS-1 cells was essentially as described (12, 13). Dishes (150 mm) containing semiconfluent monolayers of cells were rinsed with 10 ml of Dulbecco's modified Eagle's medium (DME) (Gibco Laboratories, Grand Island, NY) and incubated for 30 min with the appropriate purified plasmid DNA (20 μg in 2 ml of DME, 500 μg/ml DEAE dextran [500,000-mol-wt]) (Pharmacia Fine Chemicals, Piscataway, NJ). The cells were rinsed with 10 ml DME and then incubated for 6 h in 20 ml of DME containing 10% fetal bovine serum (Gibco Laboratories) and 100 μM chloroquine (Sigma Chemical Co., St. Louis, MO). The cells were then rinsed with 10 ml of DME and incubated an additional 40 h prior to radiolabeling. In our initial studies, we omitted the incubation in chloroquine. In later studies, however, chloroquine was included during the transfection since its presence is known to increase the percentage of transfection (14). The structure of the oligosaccharides recovered from the various VSV mutant glycoproteins was unaffected by the chloroquine treatment (relative to the same form studied in cells transfected in the absence of chloroquine) and the results are discussed using examples obtained from both transfection protocols interchangeably.

Labeling with [2-^{3}H]Mannose and Immunoprecipitation of G Protein: The growth medium from the transfected COS-1 cells was discarded and the attached cells were rinsed with 10 ml of glucose-free alpha-minimal essential medium containing 10% fetal bovine serum and 20 mM HEPES, pH 7.0 (low glucose medium). Each dish subsequently received 10 ml of low glucose medium containing 0.1 mCi/ml of [2-^{3}H]mannose (Amersham Corp., Arlington Heights, IL) (10–12 Ci/mmol). The dishes were incubated for 6 h at 37°C in a 5% CO_2 environment; 2.4 ml of low glucose medium containing 25 mM glucose and mannose was then added to each dish and an additional 2-h incubation at 37°C followed. At the conclusion of the labeling, the radioactive medium was removed and the dishes were rinsed twice with cold phosphate-buffered saline. The cells were solubilized in 3 ml of extraction buffer (25 mM HEPES, pH 7, containing 0.1 M NaCl, 0.15 trypsin inhibitory units/ml of Trasylol [Sigma Chemical Co.], 1% Triton X-100, 0.4% deoxycholate), and the extracts were clarified by centrifugation at 10,000 *g* for 20 min after a 30-min incubation on ice. The supernatants received 0.05 ml of a 10% suspension of fixed *Staphylococcus aureus* (Boehringer Mannheim Biochemicals, Indianapolis, IN) and were incubated for 20 min on ice. The bacteria were subsequently removed by centrifugation and 40 μg of affinity-purified rabbit anti-VSV G protein was added to each extract; the solutions were incubated overnight at 4°C.

The immune complexes were recovered by adding 0.05 ml of a 10% suspension of *S. aureus*, incubating them for 20 min on ice, and sedimenting the bacteria by centrifugation. The bacterial pellets were washed five times with extraction buffer containing 0.1% SDS, once with 50 mM Tris, pH 6.8, and finally suspended in 1% SDS, 1% β-mercaptoethanol, 50 mM Tris, pH 6.8, 10% glycerol. The suspensions were boiled for 3 min and clarified by centrifugation, and the supernatants were analyzed by SDS gel electrophoresis using a 10% polyacrylamide separating gel (15) and fluorography using Amplify (Amersham Corp.) as an enhancer. To recover the mutant G protein produced by COS-1 cells transfected with Δ1428, it was necessary to immunoprecipitate from the initial labeling medium since the truncated G protein is secreted from the cells (12, 13). The medium (12 ml) was adjusted to 25 mM in HEPES, pH 7, and 1% in Triton X-100 by the addition of small volumes of concentrated stock solutions, and then treated with 0.2 ml of a 10% suspension of *S. aureus*. After removal of the bacteria by centrifugation, 16 μg of anti-VSV G protein was added and the mixture was incubated overnight at 4°C. The immune complexes were recovered and analyzed as described above.

To isolate [^{3}H]mannose-labeled G protein from mature virus, COS-1 cells were infected with wild-type VSV (Indiana Biolab, Palmyra, IN) at a multiplicity of 50 plaque-forming units per cell. 1 h after infection, the cells were transferred to 10 ml of low glucose medium containing 250 μCi [2-^{3}H]mannose and incubated overnight. The supernatant fluids were collected and clarified by centrifugation at 2,000 rpm in a clinical centrifuge. The virus was collected from these fluids by centrifugation for 60 min at 30,000 rpm in a Spinco 60Ti rotor. The resulting pellet was resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, and again collected by centrifugation. The resultant virus pellet was subjected to SDS PAGE as described above.

Isolation of [2-^{3}H]Mannose-labeled Glycopeptides: The radioactive regions of the dried gel were excised and placed in 1.5 ml of 0.1 M Tris, pH 8, containing 20 mM $CaCl_2$, 50 mM β-glycerophosphate, and 10 mg/ml pronase (Calbiochem-Behring Corp., San Diego, CA); the mixtures were incubated overnight at 56°C. The digests were boiled for 3 min, diluted with 3 ml of H_2O, and clarified by centrifugation. The supernatants were recovered, dried by rotary evaporation under reduced pressure, dissolved in 1 ml of H_2O, and the glycopeptides desalted by Sephadex G-25 chromatography. The excluded [^{3}H]mannose-labeled glycopeptides were dried by rotary evaporation under reduced pressure, dissolved in 2 ml of 10 mM Tris, pH 8, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.02% NaN_3 (TBS), and applied to a Con A-Sepharose (Pharmacia Fine Chemicals) column (0.5 x 2 cm) equilibrated in TBS; the column was eluted sequentially with TBS, 10 mM α-methylglucoside in TBS, and 0.1 M α-methylmannoside in TBS at 56°C as previously described (16). Aliquots of each fraction were monitored for radioactivity, peak fractions were pooled, dried by rotary evaporation, and the glycopeptides desalted by Sephadex G-25 chromatography.

Structural Analysis of the Isolated Glycopeptides: The high mannose-type glycopeptides were dried by rotary evaporation, dissolved in

[1] *Abbreviations used in this paper:* Con A, concanavalin A; DME, Dulbecco's modified Eagle's medium; Endo H, endo-β-*N*-acetylglucosaminidase H; TBS, 10 mM Tris, pH 8, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.02% NaN_3; VSV, vesicular stomatitis virus.

0.05 ml of citrate-phosphate buffer, pH 5.4, containing 1.25 mU of endo-*N*-acetylglucosaminidase H (Endo H) (Miles-Yeda), and incubated overnight at 37°C. The digests were diluted with 2.0 ml of 2 mM Tris base and applied to QAE-Sephadex columns (0.5 x 2 cm) equilibrated in 2 mM Tris base. The columns were washed with 8 ml of 2 mM Tris base, and then eluted with 10 ml of 20 mM NaCl in 2 mM Tris base and 10 ml of 70 mM NaCl in 2 mM Tris base; in all cases, <3% of the applied radioactivity was recovered in the latter fraction and these glycopeptides were discarded.

The radioactive oligosaccharides that did not bind to QAE-Sephadex were deionized by passage over a mixed bed Amberlite MB 3 exchange resin and dried by rotary evaporation. High performance liquid chromatography of these oligosaccharides was performed using an AX-5 micropak column (Varian Associates, Inc., Palo Alto, CA) as previously described (17). The Endo H–resistant glycopeptides eluted from QAE-Sephadex with 20 mM NaCl were dried by rotary evaporation and desalted by Sephadex G-25 chromatography.

Exoglycosidase digestion of the various glycopeptides or oligosaccharides was performed as previously described (18); when used, the amount of jack bean exoglycosidase added was 0.115 mU of α-mannosidase, 0.125 mU of β-*N*-acetylglucosaminidase, and 0.014 mU of β-galactosidase. To remove sialic acid residues, we treated the glycopeptides with 2 N acetic acid for 3 h at 80°C, and then dried them by rotary evaporation under reduced pressure. After the exoglycosidase treatment, the digests (0.02 ml) were either spotted onto Whatman #1 paper (Whatman Laboratory Products Inc., Clifton, NJ) and developed by descending chromatography in ethyl acetate:pyridine:acetic acid:water (5:5:1:3) or diluted with 0.18 ml of 0.1 M ammonium bicarbonate and chromatographed on a Bio Gel P6 column (1 x 25 cm) equilibrated in 0.1 M ammonium bicarbonate.

Lentil lectin–agarose (E. Y. Laboratories, San Mateo, CA) affinity chromatography was performed as previously described (18). Methylation analysis of the [2-^{3}H]mannose-labeled glycopeptides was performed as described by Hakomori (19) and the methylated products were separated as described by Li et al. (20).

RESULTS

In the original report examining the expression of VSV mutant G proteins that possess alterations at their carboxy terminus, it was observed that many of the modifications result in the abnormal accumulation of the proteins at various subcellular locations (13). In this report, we analyze the oligosaccharides isolated from four of the most interesting molecules. The shortest of these (del-1428) lacks the cytoplasmic and transmembrane domains of the G protein; this molecule is secreted from transfected cells. The next longer molecule (del-1473) contains the transmembrane domain of the G protein but none of its cytoplasmic domain. Instead, del-1473 contains a "foreign" sequence of 12 amino acids. We chose to study del-1473 because it appeared to be restricted to the endoplasmic reticulum of transfected COS-1 cells. We also examined the structure of the oligosaccharides of del-1554, a protein which contains all but the last three amino acids of the G protein and, in addition, contains the same foreign sequence as del-1473. Rose and Bergmann (13) had shown that this foreign sequence restricts del-1554 to the endoplasmic reticulum and Golgi apparatus, preventing it from reaching the surface in 90% of the cells. When this foreign sequence is deleted from the truncated protein as in del-1554H1, the fourth molecule examined, the nearly full-length G protein reaches the surface normally (13).

Isolation of Asparagine-linked Glycopeptides

After radiolabeling transiently infected COS-1 cells with [2-^{3}H]mannose, proteins were recovered by immunoprecipitation. The precipitates were analyzed by SDS PAGE and the radioactive proteins were localized by fluorography (Fig. 1). As expected from previous studies, del-1473 and del-1428 migrate faster than the wild-type glycoprotein coded for by pSVGL (12, 13). The increase in mobility reflects the reduced size of the proteins due to deletions at their carboxy terminus.

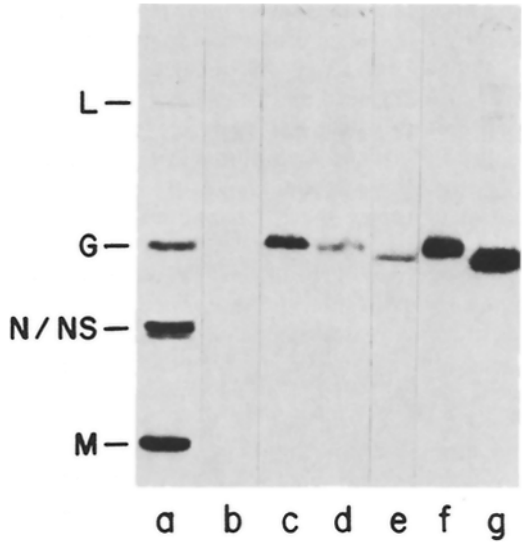

FIGURE 1 SDS gel electrophoresis of the G protein isolated from [2-^{3}H]mannose-labeled COS-1 cells. The fluorogram shows the G protein isolated by immunoprecipitation from COS-1 cells transfected with Δ1554 (lane *c*), wild-type plasmid pSVGL (lane *d*), Δ1554H1 (lane *f*), and Δ1473 (lane *g*). Del-1428 (lane *e*) was immunoprecipitated from the medium of COS-1 cells transfected with Δ1428. Lane *a* shows [^{35}S]methionine-labeled VSV standards and lane *b* shows the immunoprecipitate recovered from nontransfected [^{3}H]mannose-labeled COS-1 cells.

Del-1554 lacks three amino acid residues from the normal carboxy terminus of the G protein but possesses a twelve-residue substitute; as expected (13), this protein has a mobility on SDS gels very similar to wild-type G protein.

The radioactive regions of the dried gel were excised, the radioactivity was solubilized by pronase digestion, and the resulting glycopeptides were fractionated by several techniques. To aid the reader in following these analyses, the major fractionation procedures are outlined in Table I, along with the distribution of radioactivity recovered from the various forms of the G protein. The [^{3}H]mannose-labeled glycopeptides were first fractionated by concanavalin A (Con A)–Sepharose chromatography. As shown in Fig. 2*A*, G protein isolated from mature VSV after the infection of the COS-1 cells contains predominantly complex-type glycopeptides that either do not bind to Con A–Sepharose (Peak I; 28% of the radioactivity) or that bind and elute with 10 mM α-methylglucoside (Peak II; 68% of the radioactivity). On the basis of Con A specificity (21–24), the asparagine-linked oligosaccharides recovered in Peak I would be expected to contain tri- and/or tetraantennary structures, whereas those recovered in Con A–Sepharose Peak II should contain biantennary-type structures. A small amount of the total radioactivity (4%) isolated from the viral G protein binds tightly to Con A–Sepharose and is eluted with 0.1 M α-methylmannoside (Peak III); oligosaccharides in this fraction are presumably high mannose–type structures that were not processed to complex-type units.

The Con A–Sepharose profile of the secreted form of the G protein, del-1428, is similar to that noted above for the viral G protein; most of the radioactivity (80%) is recovered in Peaks I and II indicating that the oligosaccharides have been processed to complex-type structures (Fig. 2*B*). There is, however, a larger peak of high mannose–type glycopeptides

TABLE I. *Summary of Fractionation Procedures*

Procedure	Fraction	Composition of asparagine-linked oligosaccharides*	% of [^{3}H]Mannose-labeled radioactivity				
			Viral G protein	*del-1428*	*del-1554H1*	*del-1554*	*del-1473*
Con A-Sepharose of total glycopeptides	Peak I	Tri- and/or tetraantennary complex‡	28	17	29	8	0
	Peak II	Biantennary complex‡	68	63	64	10	3
	Peak III	High mannose‡	4	20	7	82	97
Endo H digestion of Con A-Sepharose Peak III	Sensitive	High mannose§	ND	ND	ND	70	94
	Resistant	Nongalactosylated biantennary complex§				30	6¶
Lentil lectin agarose of:							
Con A-Sepharose Peak I	Bound	Fucosylated 2,6-triantennary‖	41	65	ND	ND	NA
	Nonbound	Tri- and/or tetraantennary complex‖	59	35			
Con A-Sepharose Peak II	Bound	Fucosylated biantennary‖	77	84	ND	ND	NA
	Nonbound	Nonfucosylated biantennary‖	23	16			
Endo H resistant fraction of Con A-Sepharose Peak III	Bound	Fucosylated, nongalactosylated biantennary§	NA	NA	NA	80	NA
	Nonbound	Nonfucosylated, nongalactosylated biantennary§				20	

* The indicated compositions are based upon the results of this study (§) or upon the results of previous studies using well-characterized glycopeptides as described in references 23 and 24 (‡), and 18 and 24 (‖).
¶ The composition of this fraction was not determined.
ND and NA indicate not determined and not applicable, respectively.

(Peak III; 20% of the radioactivity) than is found in the viral G protein. Forms of the G protein that reach the cell surface (e.g., the wild-type G protein expressed from cloned cDNA and del-1554H1 [13]) possess asparagine-linked glycopeptides that fractionate virtually identically to those isolated from the viral G protein. For example, Fig. 2*C* shows the Con A-Sepharose profile of the [^{3}H]mannose-labeled glycopeptides isolated from del-1554H1; the distribution of radioactivity between Peaks I, II, and III is 29%, 64%, and 7%, respectively.

In contrast to the forms of the G protein discussed above, the two species that remain intracellular (as determined by immunofluorescence microscopy) contain a high percentage of glycopeptides that bind tightly to Con A-Sepharose and elute with 0.1 M α-methylmannoside (Peak III). In the case of del-1554 (Fig. 2*D*), 82% of the mannose-labeled radioactivity is recovered in Peak III and only 8 and 10% of the radioactivity is recovered in Peaks I and II, respectively. The predominance of Peak III is even more pronounced for glycopeptides isolated from del-1473; 97% of the mannose-labeled glycopeptides are recovered in this fraction (Fig. 2*E*).

Comparison of Complex-type Oligosaccharides Isolated from a Membrane-bound and Secreted Form of the G Protein

As noted above, the spectrum of glycopeptides isolated from del-1428 are similar to those isolated from the viral G protein. This suggests that recognition of the G protein oligosaccharides by the processing enzymes occurs independent of the protein's membrane association. To further compare the complex-type oligosaccharides associated with the secreted and membrane-bound forms of G protein, we analyzed the glycopeptides isolated in Peaks I and II from Con A-Sepharose as follows. First, the glycopeptides were fractionated by lentil lectin-agarose chromatography. Lentil lectin binds triantennary complex oligosaccharides that possess a 2,6-branch on one of the core α-mannosyl residues and an internal fucose residue attached to the *N*-acetylchitobiose core (18). Additionally, biantennary glycopeptides that are fucosylated on the *N*-acetylchitobiose unit will bind to lentil lectin-agarose (18). As shown in Fig. 3, the fraction of Con A-Sepharose Peaks I and II that bind to lentil lectin-agarose is similar for the glycopeptides isolated from the viral G protein (panels *A and C*) and del-1428 (panels *B and D*).

The second analysis examined the extent of sialylation of the two forms of the G protein. The biantennary glycopeptides (Peak II from Con A-Sepharose) were digested with the exoglycosidases α-mannosidase, β-*N*-acetylglucosaminidase, and β-galactosidase before and after mild acid hydrolysis; the acid hydrolysis step was used to selectively desialylate the glycopeptides. The digests were subsequently fractionated on a Bio Gel P6 column to separate glycopeptide-bound mannose-labeled radioactivity from mannose residues released during the exoglycosidase digestion. As shown in Table II, roughly one-third of the radioactivity is released from both the wild-type and mutant protein glycopeptides in the absence of mild acid hydrolysis. After desialylation, the amount of radioactivity released increases to >50%. Thus, the posttranslational sialylation of the biantennary oligosaccharides occurs to the same extent on the two forms of the G protein. Since omission of β-galactosidase results in very little radioactivity being released (Table II), it is also clear that the biantennary oligosaccharides of both proteins are galactosylated.

Characterization of the High Mannose Oligosaccharides Associated with del-1473 and del-1554

We expected the glycopeptides in peak III from Con A-Sepharose to contain high mannose-type oligosaccharides

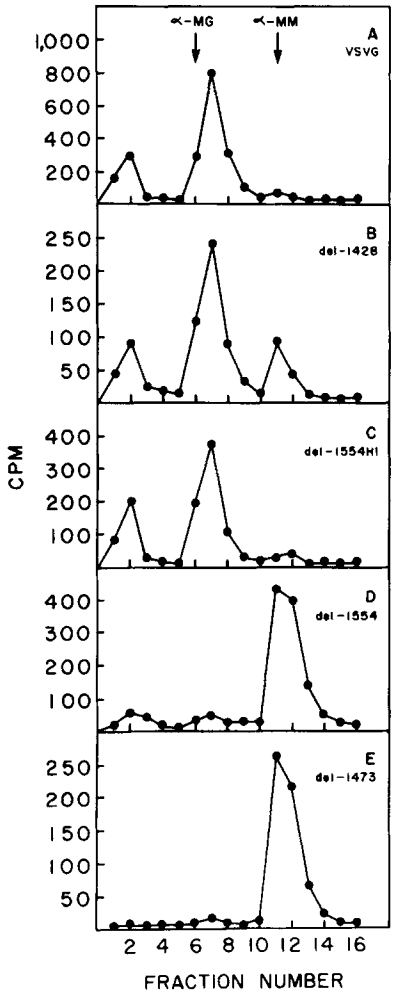

FIGURE 2 Con A-Sepharose chromatography of the [^{3}H]mannose-labeled glycopeptides isolated from the various forms of the G protein. The glycopeptides generated by pronase digestion were applied to Con A-Sepharose and the columns were eluted sequentially with TBS (peak I), 10 mM α-methylglucoside in TBS (α-MG; peak II), and 0.1 M α-methylmannoside in TBS at 56°C (α-MM; peak III). Fractions (2 ml) were collected and aliquots of each were analyzed by liquid scintillation counting. The chromatograms show the Con A-Sepharose profiles of the glycopeptides isolated from viral G protein (*A*), del-1428 (*B*), del-1554H1 (C), del-1554 (*D*), and del-1473 (*E*).

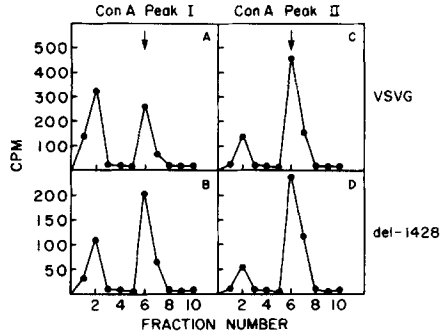

FIGURE 3 Lentil lectin-agarose chromatography of the complex-type glycopeptides isolated from a membrane-bound and secretory form of the G protein. The [^{3}H]mannose-labeled glycopeptides isolated in Con A-Sepharose peaks I and II were applied to lentil lectin-agarose columns (0.7 x 2 cm) equilibrated in TBS. The columns were washed with TBS and, at the arrow, eluted with 0.5 M α-methylmannoside in TBS; 1.5-ml fractions were collected directly into scintillation vials and counted. The chromatograms show the behavior of the Con A-Sepharose peak I glycopeptides isolated from the viral G protein (*A*) and del-1428 (*B*), and the Con A-Sepharose peak II glycopeptides isolated from the viral G protein (C) and del-1428 (*D*).

TABLE II. *Exoglycosidase Digestion of Biantennary Glycopeptides*

Treatment	% of total radioactivity released from	
	Wild-type G	*1428*
α-Mannosidase + β-N-acetylglucosaminidase	4	7
α-Mannosidase + β-N-acetylglucosaminidase β-Galactosidase	39	33
Mild acid hydrolysis + α-mannosidase β-N-acetylglucosaminidase β-Galactosidase	55	51

The [^{3}H]mannose-labeled glycopeptides derived from the viral G protein or del-1428 were treated as indicated and then analyzed by Bio Gel P6 chromatography. The radioactivity included by the column was considered released by the treatment.

that should be sensitive to digestion with Endo H; this enzyme cleaves the *N*-acetylchitobiose core of high mannose-type oligosaccharides (25). We therefore treated the peak III glycopeptides of del-1473 with Endo H and fractionated the digests by QAE-Sephadex chromatography to separate the uncharged oligosaccharides from Endo H-resistant glycopeptides. Greater than 94% of the glycopeptide-associated radioactivity is released by Endo H. However, when the same protocol is applied to the glycopeptides derived from del-1554, only 70% of the radioactivity is released. The released oligosaccharides were sized by high performance liquid chromatography. The majority of the high mannose-type oligosaccharides isolated from del-1473 contain eight mannose residues, and some possess nine, seven, and six mannose residues (Fig. 4*A*). The high mannose units isolated from del-1554 also primarily contain eight mannose residues (Fig. 4*B*).

To confirm that the high mannose-type oligosaccharides contain only mannose residues and that no other residues (such as glucose or *N*-acetylglucosamine) are present at the nonreducing termini, the Endo H-released oligosaccharides were digested with α-mannosidase. Paper chromatography of the digests (Fig. 5) indicates that both the del-1473- and del-1554-derived high mannose structures are completely degraded by the enzyme. The second peak in the chromatograms corresponds to the disaccharide mannose-*N*-acetylglucosamine which persists after α-mannosidase digestion since this mannose is in a beta linkage to *N*-acetylglucosamine (20). The ratio of radioactivity recovered as mannose to the disaccharide is 6.4:1 and 6.7:1, respectively, for the del-1473- and del-1554-derived oligosaccharides; these ratios are consistent with the observed high performance liquid chromatography profiles (Fig. 4).

Del-1554 Contains Truncated Complex-type Oligosaccharides

As noted above, 30% of the radioactivity in the Peak III glycopeptides isolated from del-1554 is resistant to Endo H.

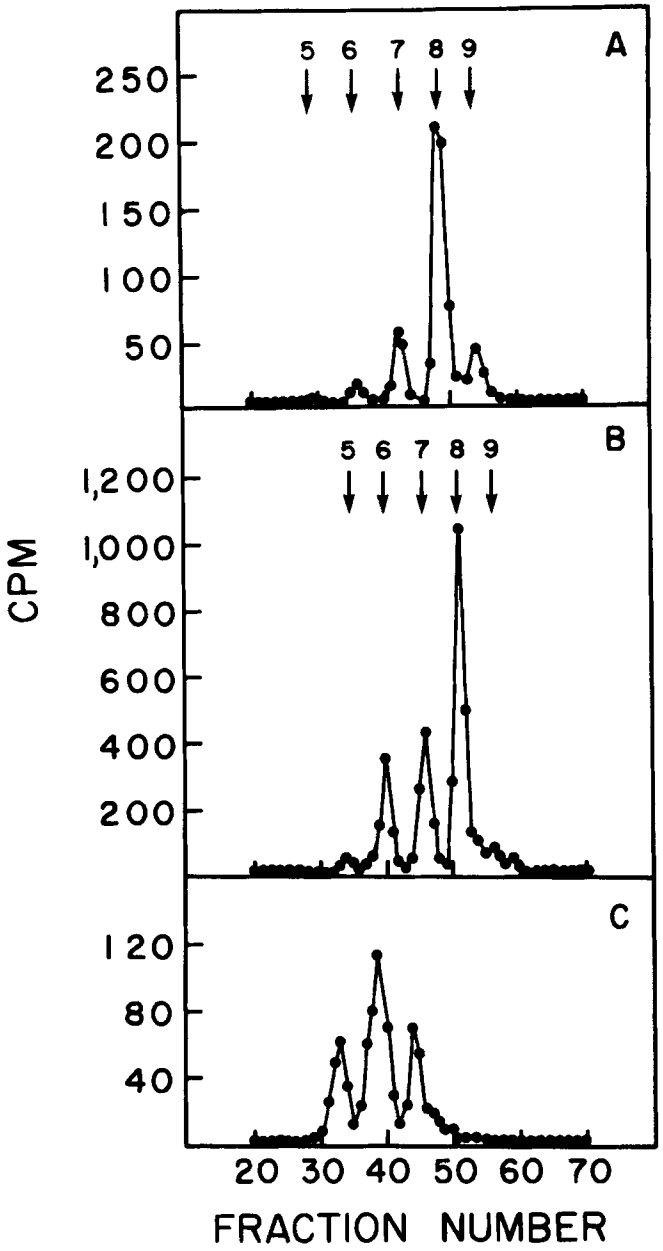

FIGURE 4 High performance liquid chromatography fractionation of [^{3}H]mannose-labeled oligosaccharides released by Endo H. The oligosaccharides were isolated from del-1473 Con A-Sepharose peak III (*A*), del-1554 Con A-Sepharose peak III (*B*), del-1554 Con A-Sepharose peak II (*C*). The elution position of high mannose oligosaccharide standards are indicated by the arrows. 5, $Man_5GlcNAc$; 6, $Man_6GlcNAc$; 7, $Man_7GlcNAc$; 8, $Man_8GlcNAc$; and 9, $Man_9GlcNAc$. The elution position of the standards in *A* is different than in *B* and *C* because the resin properties change with the age of the column.

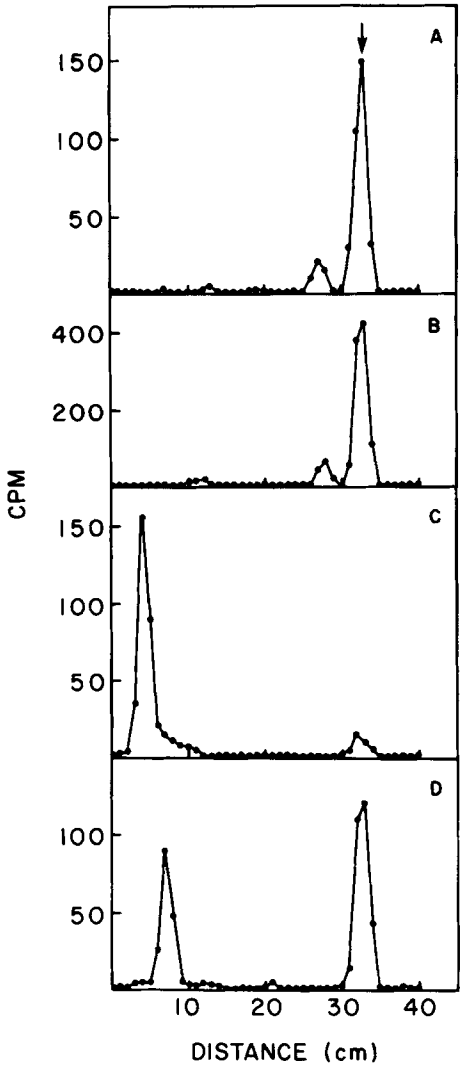

FIGURE 5 Paper chromatography of exoglycosidase digests. After incubation of the [^{3}H]mannose-labeled oligosaccharides or glycopeptides with the appropriate exoglycosidase(s), the digests were spotted on paper and the chromatogram developed by descending chromatography in ethyl acetate:pyridine:acetic acid:water (5:5:1:3). The chromatograms were cut into 1-cm strips and the radioactivity associated with each strip was determined by liquid scintillation counting. α-Mannosidase digest of Endo H-released oligosaccharides isolated from del-1473 (*A*), del-1554 (*B*). α-Mannosidase digest of the Endo H-resistant fraction of Con A-Sepharose Peak III from del-1554 in the absence (*C*) and presence (*D*) of β-*N*-acetylglucosaminidase. The arrow in *A* indicates the migration position of free mannose.

The resistant glycopeptides elute from QAE-Sephadex with 20 mM NaCl, which suggests that they contain a single net negative charge contributed by the carboxyl group of the peptide. After α-mannosidase digestion, only 9% of the radioactivity is released from the Endo H–resistant glycopeptides (Fig. 5*C*). However, inclusion of β-*N*-acetylglucosaminidase in the α-mannosidase digestion mix results in 61% of the radioactivity being released as free mannose (Fig. 5*D*). (Maximum release for a biantennary-type glycopeptide plus or minus fucose would be 50–66%.) This result, coupled with the fact that the glycopeptides were originally recovered in Peak III from Con A–Sepharose, suggested that the Endo H–resistant glycopeptides contain complex-type oligosaccharides whose antennae terminate with *N*-acetylglucosamine residues.[2]

[2] It has previously been shown that a biantennary glycopeptide possessing *N*-acetylglucosamine residues at the nonreducing termini has a fivefold higher affinity for Con A than a similar glycopeptide terminating in galactose (22). A fivefold increase in affinity could lead to a biantennary-type glycopeptide eluting in Peak III from Con A–Sepharose rather than Peak II. In addition, a biantennary-type glycopeptide (±fucose) terminating in *N*-acetylglucosamine is resistant to Endo H digestion (25).

Kornfeld et al. (18) have previously shown that fucosylated biantennary-type glycopeptides terminating in *N*-acetylglucosamine are good ligands for lentil lectin–agarose. As indicated in Table I, 80% of the radioactivity associated with the Endo H–resistant glycopeptides isolated in Con A–Sepharose Peak III binds to lentil lectin–agarose. To verify the structure of the truncated biantennary-type glycopeptides derived from del-1554, we determined the linkage pattern of the ^{3}H-labeled mannose and fucose residues by methylating the glycopeptides. As shown in Fig. 6*A*, the lentil lectin–bound fraction of the Endo H–resistant glycopeptides yields three species of methylated monosaccharide that co-migrate with 2,4-dimethylmannose, 3,4,6-trimethylmannose, and 2,3,4-trimethylfucose in a ratio of 1:1.9:1.04. This pattern is indicative of a fucosylated, biantennary-type oligosaccharide and, as shown in Fig. 6*C*, is very similar to the methylation pattern obtained for the biantennary glycopeptides isolated from the viral G protein. In this case the ratio of 2,4-dimethyl-mannose to 3,4,6-trimethylmannose to 2,3,4-trimethylfucose is 1:1.95:0.6;

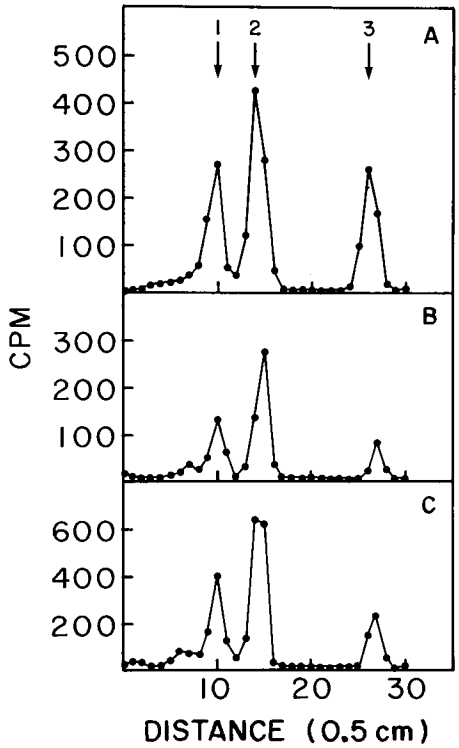

FIGURE 6 Thin layer chromatography of the ^{3}H-labeled monosaccharides obtained after methylation analysis. The acid hydrolysates generated after methylating the glycopeptides were spotted onto Silica Gel G (Analtech, Inc., Newark, DE) thin layer chromatography plates and the chromatogram developed by ascending chromatography in acetone:benzene:water:ammonium hydroxide (80:20:1.2:0.6) for 65 min. The lanes (2 cm) were divided into 0.5-cm sections and the silica gel in each section was scraped into a liquid scintillation vial and the radioactivity associated with each segment was determined. The chromatograms show the distribution of radioactivity derived from the lentil lectin-bound (*A*) and nonbound (*B*) fractions of the Endo H-resistant glycopeptides recovered in Con A-Sepharose peak III from del-1554, and the biantennary glycopeptides isolated from the viral G protein (*C*). The arrows indicate the migration position of the following methylated monosaccharide standards: 2,4-dimethylmannose (*1*), 3,4,6-trimethylmannose (*2*), and 2,3,4,6-tetramethylmannose and 2,3,4-trimethylmannose (*3*).

TABLE III. *Exoglycosidase Digestion of Complex-type Glycopeptides Isolated from del-1554*

Treatment	% of radioactivity released	
	Con A-Sepharose Peak I	Con A-Sepharose Peak II
None	0	0
α-Mannosidase	3	25
α-Mannosidase +β-N-acetylglucosaminidase	40	37
α-Mannosidase +β-N-acetylglucosaminidase +β-Galactosidase	45	43
Mild acid treatment +α-mannosidase +β-N-acetylglucosaminidase +β-Galactosidase	50	54

The [^{3}H]mannose-labeled glycopeptides isolated from del-1554 in Con A-Sepharose Peaks I and II were treated with the indicated exoglycosidase(s). After a 16-h incubation at 37°C, the digests were analyzed by Bio-Gel chromatography.

the lower amount of fucose (relative to 2,4-dimethylmannose) is due, in part, to the fact that the total Con A–Sepharose Peak II material from the virus was methylated and only 80% of these molecules are fucosylated (Fig. 3*C*).

The Endo H–resistant glycopeptides recovered in Con A–Sepharose Peak III from del-1554 that do not bind to lentil lectin–agarose again yield the same three species of methylated monosaccharides (Fig. 6*B*) in a ratio of 1:1.8:0.5. Since these glycopeptides do not bind to lentil lectin–agarose, it is likely that they do not contain fucose and that the radioactivity migrating with the 2,3,4-trimethylfucose and 2,3,4,6-tetramethylmannose markers actually corresponds to the latter compound; the two methylated derivatives do not separate under the conditions used for thin layer chromatography (20). As previously shown, α-mannosidase digestion of the total Endo H–resistant glycopeptide mixture (Fig. 5*C*) suggested that a small percentage of the oligosaccharides possess terminal mannose residues. Upon methylation, these would yield 2,3,4,6-tetramethylmannose. Thus, this fraction may contain a mixture of nonfucosylated biantennary-type structures as well as oligosaccharides that have undergone some processing (such that they are resistant to Endo H), yet contain mannose residues at nonreducing termini. This fraction contained only 5% of the total radioactivity associated with del-1554. Lack of material prevented further characterization of these structures. In total, the data indicate that the Endo H–resistant glycopeptides recovered in Peak III from Con A–Sepharose contain primarily biantennary complex-type oligosaccharides (±fucose) whose antennae terminate with *N*-acetylglucosamine.

To determine whether the complex-type glycopeptides isolated from del-1554 in Con A–Sepharose Peaks I and II also terminate with *N*-acetylglucosamine residues, these structures were digested with exoglycosidases. As shown in Table III, the

oligosaccharides in Peak I are resistant to α-mannosidase digestion. However, after digestion with both α-mannosidase and β-*N*-acetylglucosaminidase, 40% of the radioactivity is released. The further addition of β-galactosidase or pretreatment with mild acid hydrolysis and the addition of β-galactosidase each result in a net increase of 5% in the amount of radioactivity released. By virtue of their presence in Con A–Sepharose Peak I, these glycopeptides presumably contain tri- and/or tetraantennary oligosaccharides. However, as was the case with the Endo H–resistant glycopeptides recovered in Peak III, the majority of the antennae are truncated; they contain terminal *N*-acetylglucosamine residues rather than galactose and sialic acid residues.

α-Mannosidase digestion of the glycopeptides recovered in Con A–Sepharose Peak II releases 25% of the radioactivity (Table III). The addition of β-*N*-acetylglucosaminidase to the mannosidase digest increases the amount released to 37%, and 54% of the radioactivity is released after mild acid hydrolysis and treatment with β-galactosidase, β-*N*-acetylglucosaminidase, and α-mannosidase. The large release observed in the presence of only α-mannosidase suggested that the Peak II glycopeptides may contain high mannose–type glycopeptides that eluted prematurely from the Con A–Sepharose column. Indeed, Endo H digestion of the Peak II glycopeptides released 37% of the radioactivity which, when sized by high performance liquid chromatography, migrated with high mannose oligosaccharides containing five, six, or seven mannose residues (Fig. 4*C*). The fact that the high mannose units recovered in Con A–Sepharose Peak II are enriched in smaller oligosaccharides, as compared to those recovered in Peak III, suggests that mannose trimming results in a lower affinity of the glycopeptides for the lectin. Likewise, the net increase of 12% in the amount of radioactivity released by α-mannosidase in the presence of β-*N*-acetylglucosaminidase from the Peak II glycopeptides (Table III) indicates that 24% of the radioactivity in this fraction is derived from biantennary-type glycopeptides that terminate with *N*-acetylglucosamine residues; these structures, like the glycopeptides that contain smaller high mannose oligosaccharides, elute in both Peaks II and III from Con A–Sepharose. The remainder of the radioactivity in the Peak II fraction (4% of the total) is due to biantennary glycopeptides that contain galactose and sialic acid residues at the nonreducing termini.

Correcting for the fact that the high mannose oligosaccharides contain, on average, twice as many mannose residues as the Endo H–resistant glycopeptides, one can estimate that 48% of the Peak III glycopeptides isolated from del-1554 contain the truncated biantennary-type structures. In addition, 80% of the Peak I glycopeptides and 24% of the Peak II glycopeptides contain *N*-acetylglucosamine residues at the nonreducing ends of the molecules. Overall, therefore, 48% of the total oligosaccharides contain complex-type units that terminate in *N*-acetylglucosamine, 44% remain as high mannose structures, and <8% contain galactose and/or sialic acid. These latter oligosaccharides are probably derived from the 10% of the cells that express del-1554 on their surfaces (13). The percentages of the different glycopeptides isolated from Δ1554–encoded G protein have been reproducible in three separate labelings.

DISCUSSION

The introduction of specific deletions or alterations in the carboxy terminus of the G protein by site-specific mutagenesis of the DNA encoding it has previously been shown to alter the protein's subcellular localization (12, 13), suggesting that the carboxy terminus contains information necessary for the proper delivery of the newly synthesized molecules from the rough endoplasmic reticulum to the cell surface. We have made use of this system to ask two questions. (*a*) What are the structures of the asparagine-linked oligosaccharides of plasma membrane–bound and secreted forms of the G protein? (*b*) What are the structures of the asparagine-linked oligosaccharides of G proteins that fail to reach the cell surface?

Analysis of G Proteins That Are Not Blocked in Intracellular Transport

The G protein isolated from virus grown in monkey-derived COS-1 cells contains a mixture of biantennary and more-branched (tri- and/or tetraantennary) oligosaccharides, as in the case of viral G protein isolated from Chinese hamster ovary cells (26). The G protein isolated from COS-1 cells transfected with a plasmid carrying either the wild-type gene (pSVGL) or Δ1554H1 (both forms reach the cell surface) yields [^{3}H]mannose-labeled glycopeptides that fractionate on Con A–Sepharose identically to those isolated from the viral G protein. This indicates that viral infection per se does not alter the manner in which the host cell oligosaccharide-processing enzymes act upon the G protein.

A comparison of the [^{3}H]mannose-labeled glycopeptides isolated from the viral and secreted forms of the G protein indicates the following similarities. First, both proteins contain primarily biantennary-type oligosaccharides. Second, the degree to which the complex-type oligosaccharides are fucosylated, as judged by lentil lectin chromatography, is comparable for the glycopeptides recovered in both Con A–Sepharose Peaks I and II. Third, the extent to which the biantennary glycopeptides are sialylated is virtually the same, as determined by exoglycosidase digestion. These remarkable similarities between the secreted and membrane forms of the G protein suggest that both the specificity and the efficiency of the glycosidases and glycosyltransferases involved in the formation of the complex-type asparagine-linked oligosaccharides are unaffected by association of G protein with the membrane or by alterations in the protein's carboxy terminus. The only notable difference was a slight increase in the number of high mannose–type structures recovered from del-1428.

Analysis Of G Proteins That Are Blocked In Intracellular Transport

Del-1473 contains only high mannose–type oligosaccharides, consistent with its localization in the endoplasmic reticulum (13). All of the high mannose oligosaccharides isolated from the mutant protein have lost the three glucose residues from the precursor oligosaccharide. This result is expected since previous studies have shown that both glucosidase I and II reside in the endoplasmic reticulum and that glucose removal begins even before the completion of protein synthesis (27). In addition, the oligosaccharides isolated from del-1473 show evidence of mannose trimming as the major species contains eight mannose residues; this is consistent with recent reports suggesting that the endoplasmic reticulum contains α-

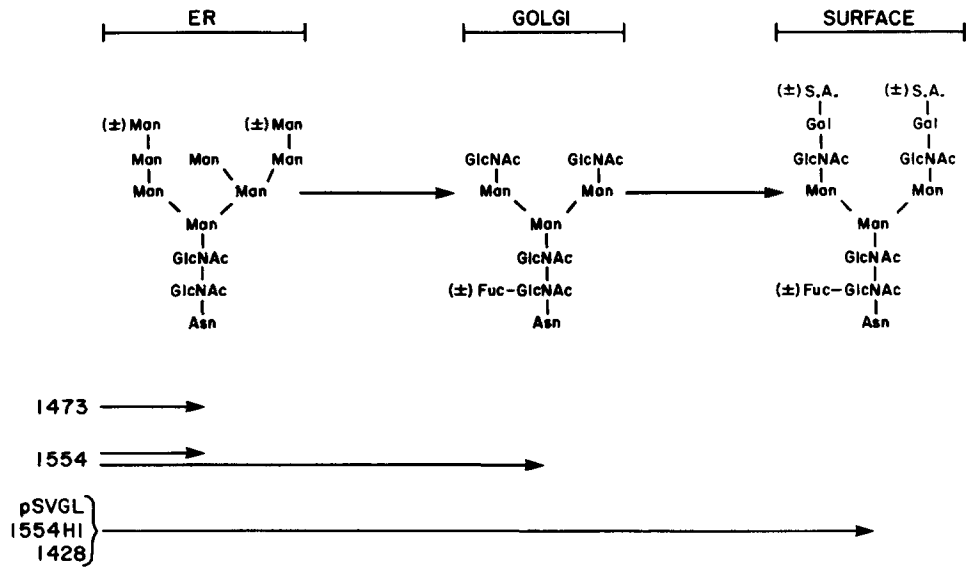

FIGURE 7 Structures of the major asparagine-linked oligosaccharides isolated from the variant VSV glycoproteins. The arrows indicate the previously assigned cellular locations of the indicated proteins (13).

mannosidase activity (27–29). It is interesting to note that the endoplasmic reticulum–associated enzyme 3-hydroxy-3-methylglutaryl-CoA reductase has previously been shown to possess high mannose oligosaccharides that contain six mannose residues (30). Since the oligosaccharides recovered from del-1473 contain a higher number of mannose residues than those isolated from the reductase, the difference in the extent of processing of the oligosaccharides on the two proteins may indicate that multiple α-mannosidases exist within distinct regions of the endoplasmic reticulum.

The most interesting results in this study are those relating to del-1554. The largest single class of glycopeptides isolated from this protein are complex-type oligosaccharides terminating in *N*-acetylglucosamine. Such molecules have been postulated as intermediates in the biosynthetic pathway for mature oligosaccharides in mammalian cells, but to our knowledge, they have not previously been isolated from wild-type cells. The oligosaccharide structural data suggest that del-1554 proteins reside at either of two separate locations: in the endoplasmic reticulum, where the asparagine-linked oligosaccharides remain as high mannose units, or in a compartment of the Golgi apparatus located proximal to the *trans* cisternae (those cisternae containing galactosyl- and sialyltransferase). An alternative explanation is that del-1544 has been transferred across the entire stack of Golgi saccules, but is in a conformation that prevents it from interacting with either galactosyl- or sialyltransferases. This appears unlikely for two reasons. First, the domain of del-1554 that differs from the G protein is on the opposite side of the membrane from the domain that contains the oligosaccharides. Second, all forms of the G protein that reach the cell surface, including the secreted form (del-1428) which totally lacks the normal carboxy terminus, have nearly identical oligosaccharides.

Fig. 7 summarizes the relationship between oligosaccharide structure and the location of the various mutant glycoproteins within the cell. The exact location of del-1554 within the Golgi apparatus is unknown and will require further morphological study for resolution. The structure of the predominant oligosaccharide, a fucosylated biantennary species terminating in *N*-acetylglucosamine residues, indicates that proteins possessing these structures have encountered the following Golgi-associated processing enzymes: *N*-acetylglucosaminyltransferases I and II, α-mannosidases I and II, and a fucosyltransferase. Previous studies, in which Golgi membranes were fractionated by sucrose density gradient centrifugation (9, 10), found that these five enzyme activities co-sediment and separate from membranes carrying an endoplasmic reticulum enzyme activity (glucosidase I) and a *trans* Golgi enzyme activity (galactosyltransferase). *N*-Acetylglucosaminyltransferase I has recently been immunocytochemically localized to a medial compartment of the Golgi apparatus (31), and it is likely that the G proteins encoded by Δ1554 that contain truncated complex-type oligosaccharides occupy a similar location. Since 80% of the del-1554 tri- and tetraantennary complex-type oligosaccharides terminate in *N*-acetylglucosamine, it is likely that the *N*-acetylglucosaminyltransferase(s) responsible for branching of the complex-type oligosaccharides also reside in a compartment proximal to that containing galactosyltransferase, as has been previously suggested (9). While the exact location remains uncertain, the data clearly indicate that proteins containing asparagine-linked oligosaccharides at intermediate stages in the formation of complex-type units can be isolated; the existence of such stable intermediates supports the notion that the Golgi apparatus is a collection of individual compartments (8–10).

We wish to thank Diamond Chan for excellent technical assistance.

This work has been partially supported by grants GM32617 and GM33342 from the National Institutes of Health.

REFERENCES

1. Struck, D. K., and W. J. Lennarz. 1980. The function of saccharide-lipids in synthesis of glycoproteins. *In* The Biochemistry of Glycoproteins and Proteoglycans W. J. Lennarz, editor. Plenum Publishing Corp., New York. 35–83.
2. Kornfeld, S., E. Li, and I. Tabas. 1978. The synthesis of complex-type oligosaccharides. *J. Biol. Chem.* 253:7771–7778.
3. Schachter, H., and S. Roseman. 1980. Mammlian glycosyltransferases: their role in the synthesis and function of complex carbohydrates and glycolipids. *In* The Biochemistry of Glycoproteins and Proteoglycans W. J. Lennarz, editor. Plenum Publishing Corp., New York. 85–160.
4. Hubbard, S. C., and R. J. Ivatt. 1981. Synthesis and processing of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 50:555–584.
5. Pollack, L., and P. H. Atkinson. 1983. Correlation of glycosylation forms with position in amino acid sequence. *J. Cell Biol.* 97:293–300.

6. Schachter, H. 1974. The subcellular sites of glycosylation. *Biochem. Soc. Symp.* 40:57–71.
7. Roth, J., and E. G. Berger. 1982. Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in *trans*-Golgi cisternae. *J. Cell Biol.* 93:223–229.
8. Dunphy, W. G., E. Fries, L. J. Urbani, and J. E. Rothman. 1981. Early and late functions associated with the Golgi apparatus reside in distinct compartments. *Proc. Natl. Acad. Sci. USA.* 78:7453–7457.
9. Goldberg, D. E., and S. Kornfeld. 1983. Evidence of extensive subcellular organization of asparagine-linked oligosaccharide processing and lysosomal enzyme phosphorylation. *J. Biol. Chem.* 258:3159–3165.
10. Dunphy, W. G., and J. E. Rothman. 1983. Compartmentation of asparagine-linked oligosaccharide processing in the Golgi apparatus. *J. Cell Biol.* 97:270–275.
11. Stanley, P. 1980. Surface carbohydrate alterations of mutant mammalian cells selected for resistance to plant lectins. *In* The Biochemistry of Glycoproteins and Proteoglycans. W. J. Lennarz, editor. Plenum Publishing Corp., New York. 161–190.
12. Rose, J. K., and J. E. Bergmann. 1982. Expression from cloned cDNA of cell-surface secreted forms of the glycoprotein of vesicular stomatitis virus in eucaryotic cells. *Cell.* 30:753–762.
13. Rose, J. K., and J. E. Bergmann. 1983. Altered cytoplasmic domains affect intracellular transport of the vesicular stomatitis virus G protein. *Cell.* 34:513–524.
14. Guan, J.-L., and J. K. Rose. 1984. Conversion of a secretory protein into a transmembrane protein results in its transport to the Golgi complex but not to the cell surface. *Cell.* 37:779–787.
15. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
16. Gabel, C. A., and S. Kornfeld. 1984. Targeting of β-glucuronidase to lysosomes in mannose 6-phosphate receptor-deficient MOPC-315 cells. *J. Cell Biol.* 99:296–305.
17. Mellis, S., and J. Baenziger. 1982. Separation of neutral oligosaccharides by high performance liquid chromatography. *Anal. Biochem.* 114:276–280.
18. Kornfeld, K., M. L. Reitman, and R. Kornfeld. 1981. The carbohydrate binding specificity of pea and lentil lectins. *J. Biol. Chem.* 256:6633–6640.
19. Hakomori, S. 1964. A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethylsulfoxide. *J. Biochem. (Tokyo).* 55:205–208.
20. Li, E., I. Tabas, and S. Kornfeld. 1978. The synthesis of complex-type oligosaccharides. *J. Biol. Chem.* 253:7762–7770.
21. Kornfeld, R., and C. Ferris. 1975. Interaction of immunoglobulin glycopeptides with concanavalin A. *J. Biol. Chem.* 250:2614–2619.
22. Baenziger, J. U., and D. Fiete. 1979. Structural determinants of concanavalin A specificity for oligosaccharides. *J. Biol. Chem.* 254:2400–2407.
23. Narasimhan, S., J. R. Wilson, E. Martin, and H. Schachter. 1979. A structural basis for four distinct elution profiles on concanavalin A-sepharose affinity chromatography of glycopeptides. *J. Biochem.* 57:83–96.
24. Cummings, R. D. and S. Kornfeld. 1982. Fractionation of asparagine-linked oligosaccharides by serial lectin-agarose affinity chromatography. *J. Biol. Chem.* 257:11235–11240.
25. Kobata, A. 1979. Use of endo- and exoglycosidases for structural studies of glycoconjugates. *Anal. Biochem.* 100:1–14.
26. Stanley, P. 1982. Carbohydrate heterogeneity of vesicular stomatitis virus G glycoprotein allows localization of the defect in a glycosylation mutant of CHO cells. *Arch. Biochem. Biophys.* 219:128–139.
27. Atkinson, P. H., and J. T. Lee. Co-translational excision of α-glucose and α-mannose in nascent vesicular stomatitis virus G protein. *J. Cell. Biol.* 98:2245–2249.
28. Hickman, S., J. L. Theodorakis, J. M. Greco, and P. H. Brown. 1984. Processing of MOPC 315 IgA oligosaccharides. Evidence for endoplasmic reticulum and *trans* Golgi α-1,2-mannosidase activity. *J. Cell Biol.* 98:407–416.
29. Bischoff, J., and R. Kornfeld. 1983. Evidence for an α-mannosidase in endoplasmic reticulum of rat liver. *J. Biol. Chem.* 258:7907–7910.
30. Liscum, L., R. D. Cummings, R. G. W. Anderson, G. N. DeMartino, J. L. Goldstein, and M. S. Brown. 1983. 3-Hydroxy-3-methylglutaryl-CoA reductase: a transmembrane glycoprotein of the endoplasmic reticulum with N-linked "high-mannose" oligosaccharides. *Proc. Natl. Acad. Sci. USA.* 80:7165–7169.
31. Dunphy, W. G., R. Brands, and J. E. Rothman. 1985. Attachment of terminal *N*-acetylglucosamine to asparagine-linked oligosaccharides occurs in the central cisternae of the Golgi stack. *Cell.* 40:463–472.