The Effect of Glycoprotein-processing Inhibitors on Fucosylation of Glycoproteins*

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The influenza viral hemagglutinin contains L-fucose linked α1,6 to some of the innermost GlcNAc residues of the complex oligosaccharides. In order to determine what structural features of the oligosaccharides were required for fucosylation or where in the processing pathway fucosylation occurred, influenza virus-infected MDCK cells were incubated in the presence of various inhibitors of glycoprotein processing to stop trimming at different points. After several hours of incubation with the inhibitors, [5,6-3H]fucose and [1-14C]mannose were added to label the glycoproteins, and cells were incubated in inhibitor and isotope for about 40 h to produce mature virus. Glycopeptides were prepared from the viral and the cellular glycoproteins, and these glycopeptides were isolated by gel filtration on Bio-Gel P-4. The glycopeptides were then digested with endo-β-N-acetylglucosaminidase H and rechromatographed on the Bio-Gel column.

In the presence of castanospermine or 2,5-dihydroxyphenyl-3,4-dihydroxypyrrolidine, both inhibitors of glucosidase I, most of the radioactive mannose was found in Glc3Man7-GlcNAc structures, and these did not contain radiouclide fucose. In the presence of deoxymannojirimycin, an inhibitor of mannosidase I, most of the [14C]mannose was in a ManαGlcNAc structure which was also not fucosylated. However, in the presence of swainsonine, an inhibitor of mannosidase II, the [3H]mannose was mostly in hybrid types of oligosaccharides, and these structures also contained radioactive fucose. Treatment of the hybrid structures with endoglycosaminidase H released the [3H]fucose as a small peptide (Fuc-GlcNAc-peptide), whereas the [14C]mannose remained with the oligosaccharide. The data support the conclusion that the addition of fucose linked α1,6 to the asparagine-linked GlcNAc is dependent upon the presence of a β1,2-GlcNAc residue on the α1,3-mannose branch of the core structure.

The study of the biosynthesis of the oligosaccharide portion of the asparagine-linked glycoproteins has been greatly facilitated by the use of inhibitors that act at specific steps in the processing pathway (1, 2). Thus, swainsonine prevents the processing of the oligosaccharide chains of the influenza viral hemagglutinin (3) and other cellular glycoproteins (4–9) by inhibiting the Golgi-processing enzyme, mannosidase II (10). Castanospermine (11, 12) and 2,5-dihydroxyphenyl-3,4-dihydroxypyrrolidine (DMDP) (13) also inhibit the processing of the viral hemagglutinin by inhibiting glucosidase I (and II). Deoxymannojirimycin inhibits the Golgi-processing mannosidase I (14, 15), but it did not inhibit the endoplasmic reticulum mannosidase or mannosidase II (16). Inhibition of the processing glycosidases alters the final structures of the oligosaccharides produced and may prevent the addition of certain modifying groups. Thus, when glucosidase I is inhibited, the major oligosaccharide structures are Glc3Man7-GlcNAc, whereas blocking mannosidase I gives rise to ManαGlcNAc structures. Finally, inhibition of mannosidase II results in the formation of hybrid types of oligosaccharides. Fig. 1 shows the structures of these processing inhibitors and indicates their site of action.

Routing of a glycoprotein through the biosynthetic pathway is due, in part, to the substrate specificities of the processing enzymes. Thus, the addition of a key glycosyl residue to an oligosaccharide can serve to convert that intermediate from a substrate to a nonsubstrate or from a nonsubstrate to a substrate, for subsequent processing enzymes in the pathway (17–19). In vitro studies of the substrate specificity of the GDP-fucose:β-N-acetylgalcosaminidase (Fuc to Asn-linked GlcNAc) α1,6-fucosyltransferase have shown that the enzyme requires a terminal β1,2-linked GlcNAc attached to the mannose residue linked α1,3 to the β1,2-linked mannose (20).

In the present study, the effects of various processing inhibitors on the fucosylation of the influenza viral hemagglutinin were examined. This study provided evidence in intact cells for the substrate specificity of GDP-Fuc:β-N-acetylgalcosaminidase (Fuc to Asn-linked GlcNAc) α1,6-fucosyltransferase. The complex oligosaccharides of the influenza virus hemagglutinin contain L-fucose in α1,6-linkage to the asparagine-linked GlcNAc residue (21). Oligosaccharides of altered structures that were produced in the presence of the various processing inhibitors showed a correlation between the presence of a β1,2-linked GlcNAc on the α1,3-mannose and their ability to be fucosylated.

EXPERIMENTAL PROCEDURES AND RESULTS

In order to determine the effects of the processing inhibitors on the fucosylation of the influenza viral glycoproteins, MDCK cells were infected with the NWS strain of influenza

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1 The abbreviations used are: DMDP, 2,5-dihydroxyphenyl-3,4-dihydroxypyrrolidine; Fuc, fucose; Endo H, endoglycosidase H.

2 Portions of this paper (including "Experimental Procedures," part of "Results," Figs. 6–8, and additional references) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. S9M-1159, cite the authors, and include a check or money order for $5.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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The inhibition of fucosylation of glycoproteins by swainsonine was studied. Cells were incubated with swainsonine and viruses were produced. Fucose and mannose were added to label the glycoproteins, and the incorporation of tritiated fucose and carbon-14 mannose was monitored.

The profiles of virus from control cells and from cells incubated in two different concentrations of swainsonine were compared. The profiles to the left of Fig. 2 are those of the initial glycopeptides, while those to the right are the glycopeptides and oligosaccharides after treatment with Endo H. The upper profile demonstrates that Endo H resolved the control glycopeptides into two quite distinct peaks with respect to the mannose label. The peak emerging first (fractions 31-40) was not susceptible to Endo H and represents the complex structures normally found in this virus. The second peak (fractions 42-50) was susceptible to Endo H as indicated by the shift in migration on the Bio-Gel columns. This peak has previously been identified as a high-mannose oligosaccharide(s).

The ratio of radioactivity in these two peaks (as [14C]mannose) was about 7:3, as found in previous studies with this virus. It should be noted from these profiles that the fucose label (i.e. 3H) was only found in the complex Endo H-resistant structures in the control virus.

It can be seen from comparing the scales of [14C]mannose and [3H]fucose in the various profiles of Fig. 2 that the incorporation of L-fucose greatly exceeded that of D-mannose. This is not surprising since the specific activity of the [14C]mannose was only 55 mCi/mmol, whereas the [3H]fucose is 64 Ci/mmol. In previous studies with this virus, we have used [2-3H]mannose with a specific activity of 10 Ci/mmol as an effective label. However, in these experiments we wanted two different isotopes for the glycopeptides, and, therefore, it was necessary to use [14C]mannose even though its incorporation was somewhat low.

The lower profiles of Fig. 2 show that as the swainsonine concentration in the medium was raised, less and less of the radioactive mannose was found in the first peak (i.e. the complex types of glycopeptides), and more of the label appeared in the Endo H-sensitive structures. Thus, at 100 ng/ml of the swainsonine, more than 70% of the radioactive mannose was in oligosaccharides that had been released by Endo H (note the change in scales in the various profiles of Fig. 2). These oligosaccharides, emerging in fractions 42-50, have previously been shown to be hybrid types of structures (3, 22). It can be seen that there was a considerable alteration in the distribution of the radioactive fucose in the swainsonine-raised viral oligosaccharides after treatment with Endo H. Thus, the amount of [3H]fucose incorporated into peak 1 decreased with increasing amounts of swainsonine, while two
new fucose-containing peaks appeared. A slow migrating peak that contained substantial amounts of $[^{14}C]$mannose, but not $[^{14}C]$mannose, emerged in fractions 53-60 and represented fucose label that had been released by the Endo H treatment. This peak emerged in the general area of the column expected for a Fuc-GlcNAc-peptide. L-Fucose has been found linked in $\alpha$1,6-bonds to the innermost GlcNAc residue of the complex chains in the influenza viral hemagglutinin (21). Since these complex chains would be expected to be altered to hybrid structures in the presence of swainsonine, Endo H would cleave between the two internal GlcNAc residues and leave the innermost GlcNAc still associated with the peptide. Some additional characterization of this fucose-containing peptide is given below. In addition, a smaller radioactive fucose peak coincided with the mannose peak in the hybrid types of oligosaccharides eluting in fractions 42-46. This radioactivity probably represents additional fucose that is linked $\alpha$1,3 to the $\beta$1,2-GlcNAc on the $\alpha$1,3-mannose branch following elongation of the branch with a galactose $\beta$1,4 residue. Such structures have been identified in the influenza viral hemagglutinin (21).

The peak containing $[^{3}H]$fucose and emerging at fractions 53-60 was pooled and subjected to amino acid and glucosamine analysis. This material did contain aspartic acid, and in fact, that was the major amino acid present. However, there was considerably more aspartic acid than there was glucosamine, indicating that this glycopeptide was contaminated with other peptides. The $[^{3}H]$fucose-labeled glycopeptide was applied to a column of Dowex 50-H+ at pH 2.0. At this pH, only the N-terminal amino acid should have a positive charge. Essentially all of the radioactivity was retained on the column and was eluted in a single peak at about 1.5 M NH4OH with a linear gradient of NH4OH. This data, combined with that given above, indicate that L-fucose is located at the innermost GlcNAc of the hybrid types of oligosaccharides and is released as a Fuc-GlcNAc-peptide by treatment with Endo H.

The various mannose-labeled and fucose-labeled products produced in the presence of swainsonine and released by Endo H were chromatographed on columns of concanavalin A-Sepharose to aid in their structural identification. The profiles obtained from the concanavalin A columns with each of these glycopeptides or oligosaccharides are shown in Fig. 3. The upper profile (profile A) is that of the complex H-resistant glycopeptides produced in the presence of swainsonine (i.e. fractions 52-42 of Fig. 2, profile F). It can be seen from this profile that some of the $^{14}H$- and $^{3}H$-labeled glycopeptides were retained on the concanavalin A columns but could be eluted with $\alpha$-methylglucoside and $\alpha$-methylmannoside. However, some of the labels also did not bind to the column and emerged in the wash. Thus, these Endo H-resistant structures appear to be a mixture of tetra-, tri-, and biantennary chains containing $[^{14}C]$mannose and $[^{3}H]$fucose (23, 24). On the other hand, the oligosaccharide chains released by Endo H from the swainsonine-raised virus (Fig. 2, fractions 43-49) were retained on concanavalin A columns and required extensive washing with 10 mM $\alpha$-methylglucoside and 100 mM $\alpha$-methylmannoside for elution. This elution pattern is demonstrated in profile B of Fig. 3. This is the expected behavior for the hybrid types of oligosaccharides. The peak eluting in the $\alpha$-methylmannoside fraction did contain small amounts of $[^{3}H]$fucose which is probably due to the $\alpha$1,3-fucose residues as indicated above. Finally, the radioactive peak emerging in fractions 54-60 of Fig. 2 was not retained on the concanavalin A columns as shown in the profile (profile C) of Fig. 3. In this case, it can be seen that a large peak of $^{3}H$ emerged in the wash. This is consistent with the anticipated behavior of a Fuc-GlcNAc-peptide.

An alternate approach to the use of the lectin affinity columns was to first fractionate the glycopeptides on the concanavalin A columns and then to treat each of the peaks that eluted from these columns with Endo H. When the entire glycopeptide fraction from the first Bio-Gel column was chromatographed on the concanavalin A columns, a peak containing both $^{14}H$ and $^{3}H$ emerged in the wash, while a large peak containing both isotopes eluted when the column was treated with 10 mM $\alpha$-methylglucoside. Another smaller peak of $^{3}H$ and $^{14}C$ was eluted when the column was treated with 100 mM $\alpha$-methylmannoside. Aliquots of each fraction were counted to determine their radioactive content.

![Fig. 3. Separation of the swainsonine-induced oligosaccharides by affinity chromatography on concanavalin A-Sepharose.](image-url)

The three oligosaccharide and glycopeptide peaks seen in Fig. 2 (profile F) were run on concanavalin A-Sepharose columns (0.8 x 4.5 cm) to aid in their identification. Profile A is that of the Endo H-resistant glycopeptide peak containing both fucose and mannose label (fractions 33-42). Profile B is that of the Endo H-released oligosaccharides containing $[^{14}C]$mannose and small amounts of $[^{3}H]$fucose (fractions 43-50 of profile F, Fig. 2). Profile C is the slower moving peak (fractions 53-60) which contains mostly $[^{3}H]$fucose. Each peak was applied to a column, and the columns were washed with the indicated buffer. Then the columns were eluted with buffer containing 10 mM $\alpha$-methylglucoside and the buffer containing 100 mM $\alpha$-methylmannoside. Aliquots of each fraction were counted to determine their radioactive content.

![Fig. 3. Separation of the swainsonine-induced oligosaccharides by affinity chromatography on concanavalin A-Sepharose.](image-url)
The swainsonine-induced glycopeptides were separated by chromatography on concanavalin A-Sepharose into "unbound" (i.e. wash) and "bound" (i.e. eluted with α-methylglucoside). Each fraction was treated with Endo H and chromatographed on the Bio-Gel P-4 column. Aliquots of each fraction were counted to determine the content of \(^{14}\text{C}\) and \(^{3}\text{H}\). Profile \(A\) is that of the wash while profile \(B\) is that of the material eluted by α-methylglucoside. Standards are as shown in Fig. 2.

The material in Fraction II was partially susceptible to Endo H and partially resistant. Thus, as seen in profile \(B\), the material emerging in fractions 33–40 contained both \(^{3}\text{H}\) and \(^{14}\text{C}\) and was resistant to Endo H. This material is probably the biantennary complex structures that are still formed because the swainsonine concentration was not high enough to completely inhibit processing to complex chains. It can also be seen from profile \(B\) that the \(^{3}\text{H}\) in Peak 1 emerged slightly ahead of the \(^{14}\text{C}\) which may indicate that the complex biantennary chains are composed of some fucosylated and some nonfucosylated species. A second peak containing both \(^{3}\text{H}\) and \(^{14}\text{C}\) also emerged from this column in fractions 41–47. This peak was sensitive to Endo H, and its elution pattern is similar to the hybrid structures seen in Fig. 2. The \(^{3}\text{H}\) in this peak probably represents α,1,3-linked fucose residues attached to outer GlcNAc residues. Finally, a third peak of radioactivity containing mostly \(^{14}\text{C}\) was released by Endo H and emerged in fractions 53–60. This peak had identical properties to the Fuc-GlcNAc-peptide peak seen in earlier experiments. The swainsonine data indicate that fucose can still be attached to the oligosaccharide even though it has not been processed to the complex type of structure. Thus, hybrid chains can still be fucosylated.

Since inhibition of glycoprotein processing at the mannose I stage did not prevent fucosylation of the protein, it was of interest to determine what effect other processing inhibitors would have on this reaction. The effect of deoxymannojirimycin on the fucosylation of the influenza viral hemagglutinin was studied to determine whether fucose could be added to high mannose oligosaccharides. Deoxymannojirimycin is an inhibitor of mannosidase I (14) and causes the formation of Man\(_{9}\)GlcNAc\(_{2}\) structures in the influenza virus (15). Thus, virus-infected MDCK cells were incubated in various concentrations of deoxymannojirimycin and then labeled with [\(^{1}\text{C}\)]mannose and [\(^{5},6\text{-H}\)]fucose. The glycopeptides were fractionated on columns of Bio-Gel P-4, before and after treatment with Endo H as shown in Fig. 5. It can be seen from the lowest profile that this inhibitor was quite effective in preventing the formation of complex chains and almost completely inhibited the incorporation of \(^{14}\text{C}\)mannose into such glycopeptides. In addition, the incorporation of \(^{3}\text{H}\)fucose was almost completely blocked in the presence of this inhibitor. Instead, at the higher concentration of deoxymannojirimycin, a single peak was observed which was almost completely susceptible to the action of α-mannosidase and gave rise to Man\(_{4}\)GlcNAc and free mannose.

The Endo H-released oligosaccharides produced in the presence of deoxymannojirimycin were chromatographed on columns of concanavalin A-Sepharose. The profile obtained is seen in the Miniprint (Fig. 5, profile C). These high-mannose oligosaccharides bound tightly to the lectin column. Elution with 100 mM α-methylmannoside released the \(^{14}\text{C}\) mannose peak which was essentially devoid of \(^{3}\text{H}\)fucose.

Fucosylation of oligosaccharides was also studied in the presence of two other processing inhibitors, castanospermine and DMDP. Castanospermine (1,6,7,8-tetrahydroxyindolizidine) is a plant alkaloid that has been shown to inhibit glycoprotein processing by acting on glucosidase I (10). The pyrrolidine alkaloid, DMDP, is another inhibitor of glucosidase I. Previous studies have shown that the oligosaccharides which accumulate in the presence of these inhibitors are GlcMan\(_{9}\)GlcNAc\(_{2}\) structures (10, 13). In the presence of each of these glucosidase I inhibitors, \(^{14}\text{C}\)mannose was incorporated into a new Endo H-sensitive oligosaccharide, whereas radioactive mannose incorporation into the Endo H-resistant structures was greatly reduced (Miniprint, Figs. 6 and 7).
Endo H-released oligosaccharides produced in the presence of castanospermine and DMDP were chromatographed on columns of concanavalin A-Sepharose (Miniprint, Fig. 8, profiles A and B). These glucose-containing high-mannose oligosaccharides required 100 mM α-methylmannnoside for elution and were devoid of [3H]fucose. Thus, the presence of glucose in the oligosaccharide, or the absence of some other signal, prevents the fucosylation of the Glc\textsubscript{Man\textsubscript{1-4}}GlcNAc\textsubscript{3} oligosaccharides.

**DISCUSSION**

The goal of this study was to use the various glycoprotein-processing inhibitors to try and determine the structural features of the oligosaccharides that define the basis for the addition of L-fucose to the chitobiosyl core region of the N-linked glycoproteins. Since the influenza virus hemagglutinin has been shown to contain L-fucose residues linked α1,6 to the innermost GlcNAc residue of the N-linked complex chains (21, 25), this system was used as a model to attempt to define the in vitro requirements for fucosylation. We were able to specifically demonstrate the following observations. 1) Since fucosylation of the hybrid chains occurred in the presence of swainsonine, the specific fucosylation of the chitobiosyl core region must not depend on the processing reactions that are associated with the α1,6-branchof the polymannose structure. 2) It appears that fucosylation of these N-linked glycoproteins requires that the oligosaccharide be previously acted on by the GlcNAc transferase I as previously postulated by Schachter and co-workers (17). This is the step in the processing pathway that precedes mannosidase II action (1). Due to lack of a processing inhibitor that specifically blocks the action of GlcNAc transferase I, the addition of fucose to Man\textsubscript{7} oligosaccharides prior to the action of this enzyme cannot be definitively ruled out. However, in the in vitro studies of Longmore and Schachter (20) these oligosaccharides were not substrates for the 6-α-fucosyl transferase from porcine liver.

3) The action of swainsonine caused the production of a fucosylated hybrid type of oligosaccharide, which was useful for showing that fucosylation on the innermost GlcNAc residue does not alter the susceptibility of the oligosaccharide to Endo H. This point has been demonstrated previously in several other studies (5, 7). It has also been recently shown with respect to sulfation of the second GlcNAc residue in the core region (26).

Arumughan and Tanzer (7) previously reported the structure of a swainsonine-modified fucobronectin oligosaccharide to be GalβGlcNAcβ-Man\textsubscript{9}-(Manα-(Manα-)-Man\textsubscript{2})GlcNAc(Fuco)-GlcNAc. Their fucosylated oligosaccharide was also susceptible to Endo H treatment. Thus, swainsonine-modified oligosaccharides whose core chitobiose region is monosubstituted with fucose appear to be digestible with Endo H. Fucosylation of other proteins in the presence of swainsonine has also been reported (5).

The influenza viral hemagglutinin also contains sulfate residues on the second GlcNAc moiety of the chitobiosyl core (21, 25). Using this model system, it was shown that sulfation of the oligosaccharide still occurred in the presence of swainsonine, but not in the presence of castanospermine. In the presence of swainsonine, the sulfated structure was a hybrid chain, and it was susceptible to the action of Endo H. However, in this case, Endo H gave an oligosaccharide that still contained sulfate and a GlcNAc-peptide that was free of sulfate.

In vitro studies on fucosylation of glycoproteins previously gave strong support to the idea that the GlcNAc residue linked β1,2 to the α1,3-mannosyl branch was a signal for fucosylation. Thus, Longmore and Schachter (20) tested various glycopeptides as fucosyl acceptors for the 6-α-fucosyltransferase from porcine liver. These in vitro studies determined that a GlcNAc in (1-2)-β linkage to the α1,3-mannose is essential for enzyme activity and that a bisecting GlcNAc attached in β1,4 linkage to the β-linked mannose prevents enzyme action. Elongation of the α1,3-antennae by the addition of Galβ1,4- or sialyl-Galβ1,4 converts the glycopeptide into an excellent acceptor for a 3-α-fucosyltransferase making the Galβ1,4(Fuco1,3)GlcNAc structure (17, 20). Such a structure probably is formed in the presence of swainsonine since we found some fucose still associated with the hybrid oligosaccharides after Endo H digestion. At any rate these studies show that the processing inhibitors can be useful tools to determine when and/or where in the processing pathway various modifications of the oligosaccharide chains occur.

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**REFERENCES**


Additional references are found on p. 14458.
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Influenza virus was raised in MDCK cells in the presence of several different concentrations of an inhibitor of glucosidase (Figure 6). The glycopeptides were then separated on columns of Biogel P-4 as shown in Figure 6 (left profile). Since this column did not resolve the complex and high-mannose structures, the entire glycopeptide peak (fractions 35-50) were pooled, digested with Endo H, and rechromatographed on the column (Figure 6, right).

The two profiles shown are those of the normal virus (control) and show two peaks of 4-carbohydrate, and only one peak of 3-carbohydrate. Thus, the two peaks found after Endo H digestion represent complex (Peak 1) and high-mannose (Peak 2) oligosaccharides. The complex structures were also labeled with 3H whereas the high-mannose were devoid of this isotope.

As shown by the upper profiles of Figure 6, in the presence of castanospermine, the incorporation of 3H-glucose into the Endo H-resistant structures was greatly reduced, whereas most of the endo-4-carbohydrate was now incorporated into a new Endo H-sensitive oligosaccharide (fractions 45-47, Profile E) that emerged from the Biogel P-4 column prior to the control high-mannose structure. This peak was only slightly sensitive to endo-2-6-sialidase digestion and stained on a calibrated Biogel P-4 column like a 3H-glucose, whereas the second peak was free of this label. The slight peak of apparent 2-3-mannosidase activity in fractions 43-45 is due to spillover from the 1H-glucosidase. Thus, the presence of glucose in the oligosaccharide, or the absence of some other essential signal, prevents the fucosylation of these oligosaccharides.

Although 3H-glucose is not as effective an inhibitor of glucosidase as 1H-glucose, it was examined as an inhibitor of fucosylation in the same type of experiments as described above for castanospermine. Figure 7 shows the profiles of the glycopeptides and oligosaccharides, before (left) and after (right) treatment with Endo H. Although the inhibition was not as extensive as that seen with castanospermine, the profiles were nevertheless similar to those seen in that experiment. Thus, the amount of radioactive material in the Endo H-sensitive peak increased dramatically at the highest 3H-glucose concentration and this peak did not contain any 1H-fucose. This peak was also partially susceptible to endo-2-6-sialidase digestion and stained like a Banes 2-3GalNAcA in the cultured biogel P-4 column (data not shown). Previous studies with this inhibitor have shown that this compound inhibits glucosidase 3H and causes the aromatization of GlcNAc2/3GalNAc structures (2).

The Endo N-released oligosaccharides produced in the presence of castanospermine, Endo H, or castanospermine + 3H-glucose were chromatographed on columns of Concanavalin A-Sepharose to analyze their carbohydrate character. Figure 8 shows the profiles obtained in each of these cases. It can be seen that almost all of the 1H-glucose in each of the oligosaccharides bound tightly to the lectin column and required 100 mM N-acetylglucosamine for elution. Almost each of these peaks was essentially devoid of 3H-fucose: the small peak of 3H at the top was apparently due to spillover from the 1H-glucose. These peaks were all of the high-mannose type and in some cases even contained glucose. However, in the case of 3H-glucose and castanospermine, a peak at 1H did bind to the lectin and emerged in the wash. These peaks emerging in the wash are probably due to contamination of the high-mannose peaks by the complex chains that are still present in the presence of the inhibitors.
Effect of Inhibitors on Fucosylation of Glycoproteins

Figure 7. The effect of DNP on the Incorporation of [1-14C]Glucosamine into VSV Glycoproteins. Incubation conditions and assay methods were the same as those described for VACV glycoproteins in Figure 2. In this experiment, DNP was added to infected cells at the concentrations shown. Standards are as shown in Figure 2.

Figure 8. Identification of O-Glycosylated VSV Glycoproteins Produced in the Presence of Various Processing Inhibitors by Chromatography on Carbohydrate-4-Sepharose. The Undigested O-glycosylated glycoproteins produced in the presence of Gentamycin B (A), DNP (B), and DM (2-deoxy-2,3-dimannose) (C) were applied to columns of Gentamycin B-Sepharose (0.8 x 4.5 cm) and the columns were washed with the starting buffer. Then, each column was washed with buffer containing 10 mM N-ethylmaleamide and then buffer containing 100 mM N-ethylmaleimide. Aliquots of each fraction were treated to determine the \(^{14}C\) and \(^{3}H\) contents.

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