The relationship between post-translational modifications of macromolecules and their intracellular routing is of fundamental importance. The availability of the indolizidine alkaloid, swainsonine, which interferes with glycoprotein processing, provides a new probe for studying relationships between glycosylation of proteins and their cellular routing. Using fibronectin as a model glycoprotein, we have explored the effect of swainsonine upon oligosaccharide structure, glycoprotein synthesis, and secretion. Confluent human fibroblasts were labeled with radioactive mannose or glucosamine in the presence or absence of swainsonine. Fibronectin was secreted into the medium of swainsonine-treated cultures and found to contain endo-β-N-acetylglucosaminidase H-sensitive oligosaccharides, instead of the normal complex endo-β-N-acetylglucosaminidase H-resistant oligosaccharides. Pronase-released glycopeptides and hydrazine-released oligosaccharides of isolated fibronectin from the culture medium were analyzed using endo-β-N-acetylglucosaminidase H and specific exoglycosidase digestions in conjunction with calibrated gel filtration chromatography. The structure of the swainsonine-modified endo-β-N-acetylglucosaminidase H-sensitive oligosaccharides was found to be a hybrid type, Galβ→GlcNACβ→Manα→[Manα→(Manα→)]Manβ→GlcNACβ→(±Fucose)→GlcNAC. Other experiments showed that the synthesis and secretion of fibronectin were not affected by its change in glycosylation. The results also infer that swainsonine inhibits Golgi mannosidase II in intact cells, and that removal of two mannose residues by that mannosidase is not a prerequisite for addition of galactose to the existing peripheral nonreducing GlcNAc or for the addition of fucose to the innermost reducing GlcNAc. The composite results indicate that significant changes in oligosaccharide structure had little effect upon the routing and cellular release of a typical N-asparagine-linked glycoprotein.

The biosynthesis of N-glycosidically linked oligosaccharides involves the initial transfer of a Glc,Man,GlcnAc, oligosaccharide chain from its lipid intermediate to the protein core (1–3). This product is believed to be the precursor of both high mannose and complex type oligosaccharides, and it undergoes trimming reactions including the removal of the 3 glucose residues and a variable number of mannose residues. In the case of complex type oligosaccharides, 4 α1,2-mannosyl residues are removed from the deglucosylated Man,GlcnAc oligosaccharide by α1,2-specific mannosidases (4, 5). This intermediate is then converted into GlcnAcMan,GlcnAc by the initial transfer of one N-acetylglucosamine by N-acetylglucosamine transferase I (6) followed by the removal of α1,3- and α1,6-mannosyl residues by α-mannosidase II (4, 5, 7, 8). The oligosaccharide synthesis is then completed by the addition of various sugar residues, catalyzed by the action of specific glycosyl transferases.

Swainsonine, an indolizidine alkaloid isolated from the plant Swainsona canescens (9) has been shown to be a potent inhibitor of lysosomal α-mannosidase (10) and produces characteristic features of α-mannosidosis in animals (11). This alkaloid was also recently isolated from the spotted locoweed Astragalus lentiginosus (12) and may be an active agent in causing locoweed in animals of the American Southwest (13). It has also been shown that swainsonine decreases the synthesis of complex type glycopeptides concomitant with a substantial increase in the high mannose type glycopeptides (14, 15). In addition, Tulsiani et al. (16) have shown that swainsonine inhibits Golgi mannosidase II but not mannosidases IA and IB and they suggested that swainsonine prevents the formation of complex glycoproteins by its inhibition of mannosidase II. More recently, Gross et al. (17) have reported that α1,antitrypsin secreted from swainsonine-treated rat hepatocytes combines features usually associated with either high mannose or complex type oligosaccharides. These studies prompted us to examine the structure of N-linked oligosaccharides of complex type glycoproteins which have been biosynthesized in the presence of swainsonine. We have used fibronectin as a prototype since it is a major complex type glycoprotein which is synthesized and secreted from fibroblasts in cell culture. We have also investigated whether swainsonine affects the synthesis and secretion of fibronectin in such cultures.

**EXPERIMENTAL PROCEDURES**

**Materials**—Swainsonine was kindly provided by Dr. Peter R. Doring, Murdoch University, Australia. [2,3H]Mannose (16 Ci/mmol), [U-14C]mannose (277 mCi/mmol), and [6-3H]glucosamine HCl (20 Ci/mmol) were purchased from New England Nuclear, while [1,14C]glucosamine HCl (56 mCi/mmol) was from ICN. Pronase was obtained from Calbiochem–Behring. Endo-H2 (Streptomyces plicatus) was purchased from Health Research Inc., NY. Exoglycosidases β-N-acetylglucosaminidase, β-galactosidase, α-mannosidase (all from jack bean), and α-L-fucosidase (bovine epidermis) were purchased from Sigma. 3H-labeled dextran was from Amersham Corp. Bio-Gel P-4 (~400 mesh) was obtained from Bio-Rad. All other chemicals used in this study were of analytical grade.

**Cell Culture and Metabolic Labeling**—Normal human skin fibroblasts (American Type Culture Collection, CRL 1493) were maintained in Dulbecco’s modified Eagle’s medium containing 100 μg/ml

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‡ To whom reprint requests should be addressed.
of ascorbate, 50 µg/ml of β-aminopropionitrile fumarate and 10% fetal calf serum. For metabolic labeling, confluent cells grown in 100-mm plastic petri dishes were rinsed with Ca²⁺Mg²⁺-free Dulbecco’s phosphate-buffered saline and incubated in 3 ml of Dulbecco’s modified Eagle’s medium containing 10% of the normal glucose concentration and 2% heat-inactivated, dialyzed fetal calf serum, with or without 0.1 µCi/ml of swainsonine as required. After 30 min of incubation, radiolabeled sugars were added at a concentration of 5 µCi/ml of [14C]-labeled sugar or 15 µCi/ml of [3H]-labeled sugar.

**Isolation Procedures**—After 6 or 12 h of incubation, medium was collected, filtered, and brought to 1 ml pepsin-methylsulfonyl fluoride, 5 mM N-ethylmaleimide and 10 mM EDTA (= protease inhibitors). For the subsequent isolation of glycopeptides, proteins were precipitated from the medium in a final concentration of 10% trichloroacetic acid and washed three times with 10% trichloroacetic acid. For the subsequent isolation of intracellular glycopeptides, cells were lysed with 1 volume of NET buffer at pH 11.0 (0.15 M NaCl, 0.05 M Tris, 0.5% Nonidet P-40, 0.2% bovine serum albumin, protease inhibitors). After 3 min at 4 °C, 4 volumes of NET buffer, pH 7.4, were added, and the total lysate was centrifuged. To the supernatant, trichloroacetic acid was added to a final concentration of 10% and centrifuged. To quantitatively remove Nonidet P-40 from the precipitate, ethanol saturated with sodium acetate was added and the precipitate resuspended and centrifuged. This procedure was repeated twice. Control studies showed removal of detergent and quantitative recovery of the proteins. Fibronectin from the medium was isolated by gelatin affinity chromatography as described elsewhere (18). Glycopeptides obtained from the total proteins or from fibronectin were generated by pronase digestion. Pronase was dissolved in 0.1 M Tris-HCl, pH 8.0, 2 mM CaCl₂, and preincubated for 1 h at 50 °C, and then digestion was carried out in the same buffer for 18 h at 50 °C with pronase at 2 mg/ml. After digestion, radiolabeled sugars were added at a concentration of 5 µCi/ml of [14C]-labeled sugar or 15 µCi/ml of [3H]-labeled sugar.

**Results**

Effect of Swainsonine on Total Glycopeptide Preparation—Gel filtration patterns of intracellular, pronase-released glycopeptides showed no discernable differences between control and swainsonine-treated fibroblasts (results not shown). Most of these glycopeptides, from both control and swainsonine samples, were found to be sensitive to endo-H digestion (not shown). When glycopeptides from the secreted glycoproteins were analyzed, it was found that the sizes of the swainsonine glycopeptides were slightly smaller than those of control glycopeptides (Fig. 1). However, a much larger proportion of the swainsonine glycopeptides were susceptible to endo-H than the control glycopeptides (Fig. 1). These results were very similar when the swainsonine concentration was increased from 0.1 to 1 or 10 µg/ml in the culture medium.

Inhibition of Formation of Complex Oligosaccharides of Fibronectin by Swainsonine—The sizes of the glycopeptides obtained from control fibronectin were slightly larger than those of swainsonine glycopeptides (not shown). Endo-H digestion of these glycopeptides showed that swainsonine glycopeptides were extensively cleaved (Fig. 2) while glycopeptides from control fibronectin were completely resistant to endo-H (not shown). Oligosaccharides were released from fibronectin by hydrazinolysis and gel chromatography of the oligosaccharides showed the swainsonine peak to be slightly smaller than the control peak (Fig. 3A). Most of the oligosaccharides from the swainsonine group were susceptible to endo-H (Fig. 3B) while those of the control group showed no change in their elution profile after digestion with endo-H (not shown).
shown). About 10% of the swainsonine oligosaccharides were not susceptible to endo-H under the conditions employed (Fig. 3B). Since endo-H does not release oligosaccharide chains that have been fully processed to the complex type (as in the case of control fibronectin), we infer that oligosaccharide processing was impaired by swainsonine treatment.

Oligosaccharide Analysis of Control and Swainsonine Fibronectin—When mannose-labeled control oligosaccharides were subjected to desialylation, a shift in the elution profile on Bio-Gel P-4 was observed (Fig. 4). A trailing shoulder has always been noted in all of the control oligosaccharides. This is consistent with our previous observations in which desialylated normal fibronectin oligosaccharides gave more than one peak when analyzed by high performance liquid chromatography (18). Moreover, in those studies, an oligosaccharide subfraction of normal fibronectin did not bind to the concanavalin A sepharose affinity column, suggesting the presence of more than one type of N-linked oligosaccharide in normal fibronectin. Endo-H digestion of swainsonine fibronectin glycopeptides. 12-h mannose-labeled fibronectin was isolated from the culture medium of swainsonine-treated fibroblasts and the pronase-released glycopeptides chromatographed before (- - -) and after (-----) digestion with endo-H. [14C]Mannose eluted in fraction 92.

FIG. 3. Gel filtration of fibronectin oligosaccharides. A, mannose-labeled oligosaccharides from control ([3H]mannose, ---) and swainsonine ([14C]mannose, - - -) samples were co-chromatographed. B, [3H]glucosamine (---) and [14C]mannose-labeled (-----) swainsonine oligosaccharides after digestion with endo-H. In preparative runs, [3H]-labeled endo-H-digested oligosaccharide samples were individually chromatographed and peaks II—IV were separately pooled from [3H]mannose and [14C]glucosamine-labeled oligosaccharides for further analysis. In this figure and in Figs. 4-7, the small arrows and numbers at the top indicate the locations of the glucose monomer and its oligomers.

Digestion of endo-H-treated swainsonine oligosaccharides with exoglycosidases gave unique results compared to the endo-H-resistant control oligosaccharides. Peak II of Fig. 3B, in mannose-labeled form, had an elution position of 10 glucose units (large arrowhead in Fig. 5A). When subjected to desialylation and β-N-acetylglucosaminidase digestion, the elution position of peak II was not altered (results not shown). Upon treatment with β-galactosidase, peak II was shifted to an elution position of 9 glucose equivalents (Fig. 5A). α-Mannosidase digestion gave two peaks (Fig. 5C), one broad peak centered between 7 and 8 glucose units and another peak corresponding to 1 glucose unit. When peak II was digested with β-galactosidase and β-N-acetylglucosaminidase, 94% of the total radioactivity was shifted to a peak of 7 glucose equivalents (Fig. 5B). α-Mannosidase digestion, along with β-galactosidase and β-N-acetylglucosaminidase, released 76% of the mannose label to the size of 1 glucose unit and 19% to the position of 3 glucose residues (Fig. 5D). When [14C]glucosamine-labeled peak II (Fig. 3B) was incubated with β-galactosidase and β-N-acetylglucosaminidase, 45% of the label was eluted at 7 glucose equivalents and 50% at 2 glucose equivalents (Fig. 6A). Digestion of peak II with these two glycosidases plus α-mannosidase caused a shift in the 7 glucose unit peak (Fig. 6A) to a 3 glucose unit peak (Fig. 6B).
Digestion of swainsonine oligosaccharides with endo-H gave two more peaks, III and IV, if the oligosaccharide had been labeled with glucosamine (Fig. 3B); with mannose-labeled oligosaccharide only peak III was obtained. Peaks III and IV correspond to elution positions of 3 and 2 glucose units, respectively. The possibility of the presence of fucose in peak III was examined by α-fucosidase digestion (Fig. 7). After α-fucosidase digestion, glucosamine-labeled peak III gave two peaks corresponding to 2 and 1 glucose units (Fig. 7A), while mannose-labeled peak III showed only one component equivalent to 1 glucose unit (Fig. 7B).

**Fig. 4.** Susceptibility of control fibronectin oligosaccharides towards desialylation and exoglycosidases. Control [3H]mannose-labeled oligosaccharides were treated as indicated and the products chromatographed. Glycosidase digestions were carried out on the desialylated oligosaccharides as indicated. The large arrow adjacent to the peak shows the elution position prior to desialylation.

**Fig. 5.** Susceptibility of endo-H-released swainsonine oligosaccharide peak II towards exoglycosidases. [3H]Mannose-labeled, endo-H-digested swainsonine peak II (Fig. 3B) was digested with glycosidases as indicated and the products chromatographed. The large arrow in A indicates the original elution position of intact peak II oligosaccharide.

**Fig. 6.** Exoglycosidase digestions of glucosamine-labeled peak II. [3H]Glucosamine-labeled peak II (Fig. 3B) was digested as shown and analyzed by column chromatography. The large arrowhead is the same as in Fig. 5.

Influence of Swainsonine on the Synthesis and Secretion of Fibronectin—Cells were labeled with [3H]proline in the presence and absence of swainsonine as described under “Experimental Procedures.” Fibronectins from the cell lysate and medium were quantitatively immunoprecipitated using rabbit antifibronectin antibody as described previously (23) except that 8 units of goat antirabbit γ globulin were added instead of Staphylococcus aureus. Results indicated (not shown) that neither the synthesis nor the secretion of [3H]proline labeled fibronectin was affected by the treatment of fibroblasts with swainsonine.

**DISCUSSION**

Fibronectin contains about 4–9.5% carbohydrate (24, 27, 28), consisting primarily of “complex” oligosaccharides having the biantennary structure (±Sia→Galβ→GlcNAcβ→Manα→Manβ→GlcNAcβ→(±Fuco)→GlcNAc linked to asparaginyl residues (20, 24, 29–31). About three to five N-linked oligosaccharides are reported to be present per fibronectin monomer (20, 31–33, 39), and most of them are con-
Swainsonine Causes Abnormal Glosylation of Fibronectin

was digested with α-fucosidase and chromatographed. The elution and presence of fucose (28-30, 32), the presence and type of concentrated on the gelatin binding site (33) of the molecule.

Variations have been noted in the carbohydrate structure: the presence of fucose (28-30, 32), the presence and type of linkage of siaic acid (29-31), and in the site-specific Galβ→GlcNAc linkages (31). The biosynthesis of fibronectin oligosaccharides appears to follow the known pathway via dolichol-linked high mannose intermediates. Olden et al. (34) observed that swainsonine prevents the formation of biantennary complex oligosaccharides of fibronectin (35, 36) indicate their conversion from the high mannose form to the complex form by intracellular processing.

Our studies of the glycopeptides derived from total secreted glycoproteins of the fibroblasts showed a slight effect of swainsonine at this level of analysis. However, the effects of the alkaloid became quite clear when the glycopeptides were analyzed following cleavage with endo-H. This observation was reinforced by the results obtained from identical studies of fibronectin glycopeptides.

After desialylation of control oligosaccharides, they eluted at a position of 14 glucose units, which could correspond to the structure of (Galβ→GlcNAcβ→Manα→)Manβ→GlcNAcβ→(±Fucα→)GlcNAc cited above. In the gel chromatography system which we used, fucose, mannose, and galactose behave as 1 glucose unit and N-acetylglucosamine as 2 glucose units (25). The series of exoglycosidase digestions, in conjunction with calibrated gel filtration, of desialylated control oligosaccharides confirms the known biantennary complex structure, and thus provides confidence in using this strategy for analysis of the swainsonine oligosaccharides.

The structure of endo-H-released swainsonine peak II (Fig. 3B), based on its elution position, percentage of label cleaved with 50% at 2 glucose equivalents, indicated that the peak at 3 glucose equivalents is Man-GlcNAc in P-linkage, since this peak was also observed when the mannose-labeled peak was treated similarly. Hence by analysis of these results, the structure of peak II is assumed to be Galβ→GlcNAcβ→Manα→[Manα→(Manα→Manβ→)]Manβ→GlcNAc.

Since endo-H digests the innermost N-acetylglucosamine of the high mannose-type oligosaccharides, peak IV (Fig. 3B), which elutes at the 2 glucose position, is probably N-acetylglucosamine. α-Fucosidase digestion of peak III (Fig. 3B) showed the presence of Fucα→GlcNAc residues. Combining all of the proposed structures of peaks II, III, and IV, the total structure of swainsonine-modified fibronectin oligosaccharide is assumed to be

$$\text{Peak I (Fig. 3B), which is endo-H resistant has not been further investigated. The proportion of this peak varied between 5-15% of the total oligosaccharide from one experiment to another.}

Elbein et al. (14, 15) showed that swainsonine prevents the formation of complex oligosaccharides and gives rise to endo-H-cleaved hexose,GlcNAc which could be composed of Manα,GlcNAc, Glcα,Man,GlcNAc and even Glc2Man,GlcNAc. However, using in vitro conditions, Tulsiani et al. (16) reported that swainsonine prevents the formation of complex glycoproteins by inhibiting Golgi mannosidase II which normally acts on GlcNAcManα,GlcNAc groups (5). The present cell culture investigation clearly shows: 1) swainsonine inhibits the formation of biantennary complex oligosaccharides of fibronectin and gives rise to a postulated hybrid type oligosaccharide Galβ→GlcNAcβ→Manα→[Manα→(Manα→Manβ→)]Manβ→GlcNAcβ→(±Fucα→)GlcNAc; 2) swainsonine apparently inhibits Golgi mannosidase II and not Golgi mannosidases IA and IB; 3) the removal of 2 mannose residues by Golgi mannosidase II is not a prerequisite for the addition of galactose to the existing peripheral nonreducing GlcNAc or for the addition of fucose to the innermost reducing GlcNAc; and 4) alteration of the complex type of oligosaccharide in fibronectin to the hybrid type does not alter the

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**Fig. 7. Fucosidase digestion of peak III.** Glucosamine (A) and mannose-labeled (B) endo-H-sensitive swainsonine peak III (Fig. 3B) was digested with α-fucosidase and chromatographed. The elution positions of untreated peak III (large arrowhead) plus the 2 glucose and 1 glucose positions are indicated.

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mannotidase digestion. As summarized in Table I, a theoretical value of 60% radioactivity should be present in the 1 glucose position for the assumed structure, whereas only 49% was found. The removal of all the peripheral α-mannose residues by α-mannotidase may be hampered by the presence of peripheral Galβ→GlcNAcβ→ residues. This is consistent with the results of Tulsiani et al. (5) in which jack bean mannosidase released only about 50% of the label from mannose-labeled GlcNAcβ→Manα→[Manα→(Manα→)]Manβ→GlcNAc. Simultaneous digestion using β-galactosidase, β-N-acetylglucosaminidase, and α-mannotidase converted 76% of the label to one glucose unit and 19% to 3 glucose equivalents, indicating that 4 mannose residues were removed from peak II by α-mannotidase digestion after the prior removal of Galβ→GlcNAcβ→ sequence. The glucosamine-labeled peak, upon digestion with β-galactosidase and β-N-acetylglucosaminidase gave two products; one with 45% of the label eluting at a position of 7 glucose units and another with 50% at 2 glucose equivalents. Digestion with β-galactosidase, β-N-acetylglucosaminidase, and α-mannotidase of glucosamine-labeled peak II gave two products corresponding to 2 and 3 glucose units, indicating that the peak at 3 glucose equivalents is Man→GlcNAc in β-linkage, since this peak was also observed when the mannose-labeled peak was treated similarly. Hence by analysis of these results, the structure of peak II is assumed to be Galβ→GlcNAcβ→Manα→[Manα→(Manα→)]Manβ→GlcNAc.

---

Since endo-H digests the innermost N-acetylglucosamine of the high mannose-type oligosaccharides, peak IV (Fig. 3B), which elutes at the 2 glucose position, is probably N-acetylglucosamine. α-Fucosidase digestion of peak III (Fig. 3B) showed the presence of Fucα→GlcNAc residues. Combining all of the proposed structures of peaks II, III, and IV, the total structure of swainsonine-modified fibronectin oligosaccharide is assumed to be

$$\text{Manα→(Manα→Manβ→)]Manβ→GlcNAcβ→(±Fucα→)GlcNAc; 2) swainsonine apparently inhibits Golgi mannosidase II and not Golgi mannosidases IA and IB; 3) the removal of 2 mannose residues by Golgi mannosidase II is not a prerequisite for the addition of galactose to the existing peripheral nonreducing GlcNAc or for the addition of fucose to the innermost reducing GlcNAc; and 4) alteration of the complex type of oligosaccharide in fibronectin to the hybrid type does not alter the

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TABLE I
Structure of endo-H-released swainsonine peak II after glycosidase digestions

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Enzyme digestion</th>
<th>Glucose equivalents*</th>
<th>Label cleaved*</th>
<th>Assumed structures of cleaved products</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]Mannose or [3H]glucosamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[3H]Mannose</td>
<td>β-Galactosidase</td>
<td>9</td>
<td>(100) 100</td>
<td></td>
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<tr>
<td>[3H]Mannose</td>
<td>α-Mannosidase</td>
<td>Peak I</td>
<td>7</td>
<td>(57) 40</td>
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<tr>
<td></td>
<td></td>
<td>Peak II</td>
<td>1</td>
<td>(49) 60</td>
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<tr>
<td>[3H]Mannose</td>
<td>β-Galactosidase</td>
<td>7</td>
<td>(94) 100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-N-Acetylglucosaminidase</td>
<td>α-Mannosidase</td>
<td>Peak I</td>
<td>3</td>
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<tr>
<td></td>
<td></td>
<td>Peak II</td>
<td>1</td>
<td>(76) 80</td>
</tr>
<tr>
<td>[3H]Glucosamine</td>
<td>β-Galactosidase</td>
<td>7</td>
<td>(45) 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-N-Acetylglucosaminidase</td>
<td>α-Mannosidase</td>
<td>Peak I</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Peak II</td>
<td>2</td>
<td>(50) 50</td>
</tr>
<tr>
<td>[3H]Glucosamine</td>
<td>β-Galactosidase</td>
<td>7</td>
<td>(45) 50</td>
<td></td>
</tr>
<tr>
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<td>β-N-Acetylglucosaminidase</td>
<td>α-Mannosidase</td>
<td>Peak I</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peak II</td>
<td>2</td>
<td>(50) 50</td>
</tr>
</tbody>
</table>

*Glucose equivalents were calculated based on the elution profile of the labeled peak and the theoretical value for the assumed structure.

Calculations were based on assumed structures. Values in the parentheses were calculated from the experiment.

Per cent distribution was not calculated due to incomplete separation of peak I and II (Fig. 6B).

We also report cellular fucosylation of hybrid type oligosaccharides. It is interesting to note that both [2-3H]mannose and [6-3H]glucosamine labeled fucose significantly. It is known that mannose may be converted into other monosaccharides including fucose (38). Our results suggest that glucosamine may also be converted to fucose under the conditions employed in this study. Recently Longmore and Schachter (39) have shown that GlcNAcMan,GlcNAc,Fuc glycopeptide acts as a substrate for the Golgi fucosyl transferase isolated from porcine liver and converts it into GlcNAc-Man,GlcNAc,Fuc glycopeptide in vitro. A similar fucosylation pattern has been obtained for the oligosaccharides of &alpha;1-antitrypsin secreted from swainsonine treated hepatocytes (17). Most of the hybrid type oligosaccharides contain an extra &beta;-N-acetylglucosamine residue at the C-4-positions of the &beta;-mannose residue. Perhaps, for rhodopsin, a pathway of incomplete processing by &alpha;-mannosidase II is followed by events leading to the formation of the unique rhodopsin oligosaccharides.
Swainsonine Causes Abnormal Glycosylation of Fibronectin

In animals, swainsonine or swainsonine-containing plants induce a lysosomal disorder, biochemically and morphologically similar to that of human a-mannosidosis (11, 43). Our cell culture results do not directly account for the cytologic observations, since our fibroblast seemed normal when viewed by phase contrast microscopy. However, considerations of swainsonine concentration, exposure time, cell type, and other factors may be important for emulating the phenomena found in animals which ingest the toxic alkaloid. For example, receptor-mediated uptake of abnormally glycosylated glycoproteins, followed by incomplete lysosomal digestion, might provide at least one basis for the in vivo observations. In the fibroblast cultures it does not appear that the abnormally glycosylated fibronectin follows such a pathway and other types of cells, such as macrophages, may provide a more suitable test system for examining this postulate.

Acknowledgment—We thank Dr. Peter R. Dorling, Murdoch University, Australia for kindly providing swainsonine.


REFERENCES


Abnormal glycosylation of human cellular fibronectin in the presence of swainsonine.

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