Swainsonine Causes the Production of Hybrid Glycoproteins by Human Skin Fibroblasts and Rat Liver Golgi Preparations*

Daulat Ram P. Tulsiani and Oscar Touster

From the Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

The synthesis of glycoproteins containing N-linked complex oligosaccharides is blocked by swainsonine at the step catalyzed by Golgi mannosidase II (Tulsiani, D. R. P., Harris, T. M., and Touster, O. (1982) J. Biol Chem. 257, 7936–7939). Accordingly, hybrid glycoproteins might be produced in the presence of swainsonine. In this report, we demonstrate that swainsonine causes human skin fibroblasts to synthesize such glycoproteins. In control fibroblasts, there were approximately equal amounts of complex and high mannose glycoproteins. In the presence of swainsonine (10 μg/ml), most of the complex glycoproteins were replaced by hybrid types. The principal oligosaccharide had the following structure:

\[
\text{Man}^\alpha\beta\text{Man}\overset{\beta}{\text{Man}}\overset{\alpha}{\text{Man}}\text{GlcNAc}
\]

A smaller amount of the asialo hybrid was also produced. The structure of the hybrid was established by Bio-Gel P-4 fractionation of oligosaccharides produced by endoglycosidase II treatment of pronase-derived glycopeptides, followed by examination of the susceptibility of the oligosaccharide to glycohydrolases and by its adsorbability to serotonin-Sepharose 4B. The same hybrid oligosaccharide was produced efficiently by rat liver Golgi membranes in the presence of [\(^3\)H]Man\(_n\)GlcNAc, UDP-GlcNAc, UDP-Gal, CMP-NeuAc, and swainsonine. Golgi mannosidase II had no action on the hybrid oligosaccharide, and little action on asialo hybrid, but both were converted to the mannosidase treated with neuraminidase and \(\beta\)-galactosidase. Jack bean \(\alpha\)-D-mannosidase gave the expected yields of free mannose from the various oligosaccharides studied in this work. Swainsonine should be useful in investigating the role of oligosaccharide structure of glycoproteins because of its ability to alter the oligosaccharide.

The biosyntheses of glycoproteins containing asparagine-linked high mannose and complex oligosaccharides initially follow a common pathway which then diverges to produce the two classes of glycoproteins (1–6). When \(\text{Man}_n\) derivatives have been converted to \(\text{Man}_{n+2}\) derivatives by \(\alpha_1-2\)-mannosidases (7, 8), the latter are \(N\)-acetylglucosaminylated to form GlcNAcMan\(_n\) species. Golgi mannosidase II then removes the two terminal \(\alpha_1-3\)- and \(\alpha_1-6\)-mannosyl residues to yield GlcNAcMan\(_n\) derivatives (7–10), which are then converted by several glycosyltransferases to complex oligosaccharides. We recently reported that swainsonine is a potent inhibitor of Golgi mannosidase II and blocks mannosyl cleavage at the GlcNAcMan, stage (11). Since this oligosaccharide possesses the region of human immunoglobulin D (18), avian myeloblastosis-associated viruses (19), and Prague C Rous sarcoma glycoproteins (20).

We now report that human skin fibroblasts partially synthesize glycopeptides containing oligosaccharides of the hybrid type instead of complex oligosaccharides when the cells are cultured in the presence of swainsonine. Similar products are produced in vitro when rat liver Golgi membranes are incubated with appropriate substrates in the presence of the inhibitor.

EXPERIMENTAL PROCEDURES

Materials—Male Wistar rats (125–150 g) were from Harlan Industries, Inc. Swainsonine isolated from Rhizoctonia leguminicola (21) was provided by Drs. H. P. Broquist and T. M. Harris of Vanderbilt University. Uniformly labeled oligosaccharides were provided by Drs. S. C. Hubbard and P. W. Robbins of Massachusetts Institute of Technology and were prepared as described (8). Other chemicals were obtained from the following sources: Bio-Gel P-4 (400 mesh), Bio-Rad Laboratories; Pronase-CB protease, Calbiochem-Behring Corp.; Endo H, \(^1\)Miles Laboratories, Inc.; Clostridium perfringens neuraminidase, Bectaesda Research Laboratories; \(\text{p}^2-[\text{H}]\)-mannose, New England Nuclear; serotonin (5-hydroxytryptamine-creatin sulfate complex), ICN Pharmaceuticals Inc.; UDP-GlcNAc, UDP-Gal, CMP-NeuAc, \(\beta\)-galactosidase, and \(\text{N-acetyl}\beta\)-D-glucosaminidase, Sigma. Serotonin-Sepharose 4B affinity resin was prepared as described (22). The preparation of rat liver Golgi membranes was based on the method of Leelavathi et al. (23) as described by Tulsiani et al. (24). These highly purified Golgi membranes were washed with 0.4 M NaCl to remove adsorbed proteins. Homogeneous preparations of Golgi mannosidase II was obtained as described previously (24).

Cell Culture—Diploid human skin fibroblasts (repository No. GM2936A) were obtained from the Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, NJ. The cells were maintained in 75-cm\(^2\) Falcon flasks in Ham's F-12 medium containing 5% fetal calf serum.

Labeling of Fibroblast Glycoproteins in the Presence and Absence of

---

* This investigation was supported in part by Grant GM 26430 and by Biomedical Research Support Grant S07-RR07201 from the National Institutes of Health, United States Public Health Service. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Swainsonine—Cells were grown to a density of 1.0 to 1.2 mg of cell protein/flask. They were then carefully rinsed with 5 ml of Waymouth MAB 67/3 medium, as formulated in the catalogue of Gibco (Grand Island, N.Y.), with 5% dialyzed fetal serum and 5 mg of glucose/ml medium. The flasks were divided into two groups. The medium in control flasks was replaced with 5 ml of fresh medium (see above). The medium in experimental flasks was replaced by 5 ml of fresh medium supplemented with 10 μg of swainsonine/ml of medium. After 24 h at 37 °C, the culture medium from control and experimental flasks was removed by aspiration, and the cells were rinsed twice with 5-ml portions of glucose-free medium. Each flask of the control cells was mixed with 5 ml of the above Waymouth medium containing 5% dialyzed fetal calf serum, 0.1 mg of glucose/ml of medium, and 0.1 μCi of [2-3H]mannose/ml of medium. Each flask of the experimental cells was mixed with 5 ml of the same medium per flask supplemented with 10 μg of swainsonine/ml of medium. The cells were incubated for 24 h at 37 °C.

Harvesting of Cells—The cells from control and experimental culture flasks were detached by trypsinization and collected in tubes by centrifugation at 400 × g for 3 min. The cells were washed twice with 6- and 2.5-ml portions of cold 0.9% NaCl containing 1% bovine serum albumin and twice with 5-ml portions of 0.25 M sucrose in 10 mM Tris Cl, pH 8.0.

Preparation and Pronase Digestion of Cellular Glycoproteins—The cells collected after the second sucrose wash were dried in a Bio-Dryer and extracted three times with 1.5-ml portions of chloroform/methanol/water (10:10:3, v/v/v). The insoluble residue, dried under N2, contained labeled glycoproteins (25). The residue from each flask was suspended in 0.2 ml of 100 mM Tris-Cl, pH 7.5, containing 10 mM CaCl2. Pronase-CB protease (2 mg) was added to each tube, and the mixtures were incubated for 37 °C under a few drops of toluene for 3 days, with additions of pronase (1 mg) after 24 h and 48 h. The mixture was inactivated by heating the mixture at 100 °C for 5-7 min, and the labeled glycopeptides were fractionated on a column of Bio-Gel P-4.

Endoglycosidase H Treatment of Glycopeptides—The [3H]mannose-labeled glycopeptide fractions were pooled, evaporated to dryness, suspended in 1 ml of distilled water, and dried again. Finally, the glycopeptides were suspended in 25 μl of 100 mM sodium citrate buffer, pH 5.5, containing 0.27% sodium azide and incubated with 0.025 unit of Endo H in a total volume of 50 μl. After 24 h at 37 °C, additional Endo H (0.025 unit) was added, and the incubation was carried out for an additional 24 h at 37 °C. At the end of the incubation, the reaction mixture was inactivated by heating at 100 °C for 5-7 min, and the oligosaccharides were fractionated on a column of Bio-Gel P-4.

Exoglycosidase Digestion of Oligosaccharides—Jack bean exo-β-N-acetylglycosaminidase and α-mannosidase as well as Golgi mannosidase II digestion were carried out as described previously (8). Jack bean β-d-galactosidase digestion was carried out in a total volume of 50 μl containing 100 mM sodium citrate buffer, pH 3.5, and 0.25 unit of enzyme. Additional unit was added after 18 h at 37 °C, and the incubation was carried out for an additional 8 h at 37 °C. Neuraminidase digestions were carried out for 24 h at 37 °C in a total volume of 50 μl containing 100 mM sodium acetate buffer, pH 5.5, and 0.25 unit of enzyme.

Protein was assayed by the fluorometric method of Anderson and Desnick (26) using bovine serum albumin as standard.

RESULTS

Production of Hybrid Oligosaccharides by Rat Liver Golgi Preparations in the Presence of Swainsonine—We have reported that the ([3H]Man)GlcNAc and ([3H]Man)GlcNAC incubated with Golgi membrane suspensions in the presence of UDP-GlcNAc and swainsonine are converted to GlcNAc([3H]Man)GlcNAc, whereas in the absence of the alkaloid, the products were GlcNAc([3H]Man)GlcNAc and GlcNAC([3H]Man)GlcNAC (11). In the studies reported below, ([3H]Man)GlcNAc was (a) incubated with Golgi membrane suspension in the presence and absence of 10 μM swainsonine and nucleotide sugars for various time periods, (b) the reaction mixtures were fractionated on Bio-Gel P-4 columns, and (c) the structures of isolated oligosaccharide products inferred from their elution positions were subjected to confirmation by digestion of the oligosaccharides with specific glycohydrolases.

When ([3H]Man)GlcNAc was incubated with Golgi membrane suspension in the absence (Fig. 1A) and presence of 10 μM swainsonine (Fig. 2A), nearly all the oligosaccharide was recovered unchanged from the P-4 column. Swainsonine had no effect on the processing of this oligosaccharide unless UDP-GlcNAc was also present. In the absence of swainsonine, this nucleotide caused this production of free [3H]mannose and GlcNAc([3H]Man)GlcNAc (Peak 1, Fig. 1B). In the presence of swainsonine, the release of free [3H]mannose was blocked due to inhibition of Golgi mannosidase II; this inhibition caused the accumulation of GlcNAc([3H]Man)GlcNAC (Peak 2, Fig. 1B). The presence of UDP-Gal in the incubation mixture containing ([3H]Man)GlcNAc, UDP-GlcNAc, Golgi membrane suspension, and swainsonine caused the production of two oligosaccharides. Peak 1 oligosaccharide (Fig. 2C) appears to be GalGlcNAc([3H]Man)GlcNAc on the basis of (a) its elution from the P-4 column (elutes at position expected of ([3H]Man)GlcNAc because GlcNAc has the effect of 2 mannosyl residues) and (b) treatment with jack bean mannosidase.

![Figure 1](http://www.jbc.org/) Fractionation of products formed by the action of Golgi membranes on ([3H]Man)GlcNAc; effect of nucleotide sugars. The reaction mixtures contained ([3H]Man)GlcNAc (~20,000 cpm), 100 mM Na cacodylate buffer, pH 6.2, 5 mM MgCl2, 5 μM of Golgi membrane suspension (8.0 mg of membrane protein/ml of 0.5% Triton X-100), and additions as indicated below. After incubation for 8 h at 37 °C, the reactions were stopped by heating at 100 °C for 5-7 min, and each mixture was applied to a Bio-Gel P-4 (1 × 214 cm, -40 mesh) equilibrated with 0.1 M acetic acid. The column was eluted with 0.1 M acetic acid at a flow rate of 1.8 ml/h. Fractions (0.6 ml) were collected, and the radioactivity was measured in aliquots as described (8). The column exclusion volume (V0) was determined using bovine serum albumin. The standards are GM3N, Glc(Mann)GlcNAc; GM2N, Glc(Mann)GlcNAc; GM1N, ManGlcNAc; GM0N, ManGlcNAc; GM2N, ManGlcNAc; GM1N, ManGlcNAc; GM0N, ManGlcNAc; GM1N, ManGlcNAc; GM0N, ManGlcNAc; A, mannose; B, same as A plus 10 mM UDP-GlcNAc; C, same as B plus 10 mM UDP-Gal; D, same as C plus 10 mM CMP-NeuAc.
nosidase, which released 49% of the label as free mannose. It is interesting that GalGlcNac([3H]Man)5GlcNAc, like GlcNac([3H]Man)5GlcNAc, like GlcNac([3H]Man)5GlcNAc, is sensitive to jack bean mannosidase. However, unlike the latter, it is resistant to Golgi mannosidase (Table I), a result suggesting that terminal GlcNAc is required for an oligosaccharide to be cleaved by Golgi mannosidase. Treatment of Peak 1, Fig. 2C, oligosaccharide with jack bean β-galactosidase resulted in its elution from a P-4 column at the position expected of GlcNac([3H]Man)5GlcNAc. This modified oligosaccharide is now sensitive to both jack bean mannosidase and Golgi mannosidase II (Table I).

The presence of CMP-NeuAc in the incubation mixture of Fig. 2C caused the production of three oligosaccharides (Fig. 2D). Peak 1 oligosaccharide has the expected hybrid structure, NeuAcGalGlcNac([3H]Man)5GlcNAc, as indicated by (a) its elution position from a P-4 column, (b) its susceptibility to jack bean mannosidase but resistance to Golgi mannosidase II (Table I), and (c) study of its degradation by neuraminidase and β-galactosidase. These enzymes cleaved sialyl and galactosyl residues, respectively, and the resulting oligosaccharide eluted from P-4 column at the position expected of GlcNac([3H]Man)5GlcNAc. This oligosaccharide was hydrolyzed by both jack bean mannosidase and Golgi mannosidase II (Table I). Peak 2 and Peak 3 oligosaccharides in Fig. 2D have been characterized as GalGlcNac([3H]Man)5GlcNAc and GlcNac([3H]Man)5GlcNAc, respectively (Table I). Table II shows effect of swainsonine on the in vitro processing of high mannose oligosaccharide as a function of time. It is interesting that when ([3H]Man)5GlcNAc was incubated with Golgi membranes in the presence of UDP-GlcNAc and swainsonine, nearly all of the oligosaccharide was converted to GlcNac([3H]Man)5GlcNAc in 2 h. Addition of UDP-Gal caused the formation of 11 and 36% GalGlcNac([3H]Man)5GlcNAc in 2 and 24 h, respectively. However, addition of CMP-NeuAc caused the reaction to proceed much faster. The nucleotide caused the formation of 30 and 66% NeuAcGalGlcNac([3H]Man)5GlcNAc in 2 and 24 h, respectively (Table II). Therefore, under the conditions used, GlcNac- and sialyltransferases act more rapidly than Galtransferase.

The processing of ([3H]Man)5GlcNAc in the absence of swainsonine but presence of UDP-GlcNAc and UDP-Gal (Fig. 1C) caused the oligosaccharide products to elute in several peaks. These oligosaccharides have not been extensively identified but appear to be complex type, since jack bean mannosidase treatment released a negligible amount of free mannose (~5%). Similarly, the further addition of CMP-NeuAc (Fig. 1D) caused the ([3H]Man)5GlcNAc products to elute in several peaks which have not been characterized. However, their resistance to jack bean mannosidase suggests that they are also complex-type oligosaccharides.
TABLE 1
Susceptibility to mannosidases of [3H]mannose-labeled processing intermediates produced by rat liver Golgi membranes

The oligosaccharides obtained from the peak fractions shown in Fig. 2 (indicated by the bars) were pooled and dried in a Bio-Dryer; the residues were suspended in H2O and redried. The residues were suspended in 25 μl of H2O and aliquots were used for the enzymatic digestions.

| Isolated oligosaccharide | Mannosidase treatment* | Assigned structure |
|--------------------------|------------------------|--------------------|
|                          | Jack bean mannosidase  |                    |
|                          | Golgi mannosidase I1    |                    |
| %[3H]mannose released    |                        |                    |
| Peak 1, Fig. 2A          | 74.8 (60.0)            | Man → Man                      |
|                          | 1.0 (0.0)              | Man-α1,6-GlcNAc             |
|                          |                        | Man-α1,3-Man-α1,6-GlcNAc     |
| Peak 1, Fig. 2B          | 48.8 (60.0)            | Man-α1,6-GlcNAc             |
|                          | 34.6 (40.0)            | Man-α1,6-Man-β-GlcNAc        |
|                          |                        | GlcNAc-Man-α1,6-GlcNAc       |
| Peak 1, Fig. 2C*         | 47.7 (60.0)            | 6.1 (?)                      |
|                          |                        | Man-α1,6-GlcNAc             |
|                          |                        | Man-α1,3-Man-β-GlcNAc        |
|                          |                        | GlcNAc-Man-α1,3-GlcNAc       |
| Peak 2, Fig. 2C          | 48.3 (60.0)            | 32.2 (40.0)                 |
|                          |                        | Man-α1,6-GlcNAc             |
|                          |                        | Man-α1,6-Man-β-GlcNAc        |
|                          |                        | GlcNAc-Man-α1,6-GlcNAc       |
| Peak 1, Fig. 2D*         | 47.6 (60.0)            | 2.2 (?)                      |
|                          |                        | Man-α1,6-GlcNAc             |
|                          |                        | Man-α1,6-Man-β-GlcNAc        |
|                          |                        | NeurAc-Gal-GlcNAc-Man-α1,3-GlcNAc |
| Peak 2, Fig. 2D*         | 47.8 (60.0)            | 6.6 (?)                      |
|                          |                        | Man-α1,6-GlcNAc             |
|                          |                        | Man-α1,6-Man-β-GlcNAc        |
|                          |                        | Gal-GlcNAc-Man-α1,3-GlcNAc   |
| Peak 3, Fig. 2D          | 49.2 (60.0)            | 32.4 (40.0)                 |
|                          |                        | Man-α1,6-GlcNAc             |
|                          |                        | Man-α1,6-Man-β-GlcNAc        |
|                          |                        | GlcNAc-Man-α1,6-GlcNAc       |

* Incubations were carried out at 37 °C for 24 h as described (11). Theoretical values for the assigned structures are in parentheses.

If the oligosaccharide was incubated with jack bean β-galactosidase (0.5 unit) for 24 h at 37 °C and applied to a P-4 column, the product eluted from the column at the position expected of GlcNAcManα1,6-Man-β-GlcNAc. The modified oligosaccharide binds to the serotonin-Sepharose 4B affinity column (see Table IV). When treated with neuraminidase for 24 h at 37 °C and applied to a P-4 column, it elutes from the column at the position expected of GalGlcNAcManα1,6-Man-β-GlcNAc. The modified oligosaccharide does not bind to the serotonin-Sepharose 4B column.

mannosidase II showed negligible activity toward the sialylated and asialo hybrids, but, as expected, this enzyme removed 2 mannosyl residues from GlcNAc(α1,6)[3H]Man3GlcNAc (~34% [3H]mannose of 40% theoretically possible (Fig. 3B)). This experiment again indicated the substrate specificity of Golgi mannosidase II. Treatment of sialylated hybrid with neuraminidase and then with β-galactosidase, or treatment of asialo hybrid with β-galactosidase, yielded oligosaccharide which eluted from a P-4 column at the position expected of GlcNAc(α1,6)[3H]Man3GlcNAc. As expected, this oligosaccharide was sensitive to both the jack bean mannosidase and Golgi mannosidase II.

Production of Hybrid Glycoprotein by Human Skin Fibroblasts in the Presence of Swainsonine—Fibroblasts were labeled with [3H]mannose and their glycopeptides prepared and fractionated as described under "Experimental Procedures." The control and swainsonine-treated cells incorporated similar amounts of [3H]mannose. The elution profiles of [3H]mannose-labeled glycopeptides of the control (Fig. 4A) and swainsonine-treated cells (Fig. 4D) were quite similar, with the exception of a larger radioactive peak between fractions 120 and 140 of the swainsonine-treated cells. However, when the glycopeptides were treated with Endo H and oligosaccharide products were fractionated on a Bio-Gel P-4 column, the
Effect of Swainsonine on Glycoprotein Biosynthesis

Oligosaccharide profiles of control cells (Fig. 4A) and swainsonine-treated cells (Fig. 4D) were quite different. Nearly 35% of the label in glycopeptides from control was unchanged by Endo H treatment (fractions 101-140, Fig. 4A) and therefore apparently contained complex-type oligosaccharide. Resistance to Endo H was also demonstrated by treating fractions 161-140, Fig. 4A, with the enzyme and observing that the position of elution of radioactivity from a P-4 column was unchanged (Fig. 4B). As expected for complex oligosaccharide, the eluted material was resistant to jack bean mannosidase.

**Table II**

Effect of incubation time on the production of oligosaccharides by Golgi membranes in the presence of swainsonine and various nucleotide sugars

| Incubation time (h) | Additions | M,M | M,N | M,N,M | GalNM,M | SGalNM,M |
|---------------------|-----------|-----|-----|-------|---------|---------|
| 2                   | None      | <2.0| 98.0| None  | None    | None    |
|                     | UDP-GlcNAc| <2.0| 8.0 | 90.0  | None    | None    |
|                     | UDP-GlcNAc + UDP-Gal | <2.0| 7.6 | 79.2  | 11.2    | None    |
|                     | UDP-GlcNAc + UDP-Gal + CMP-NeuAc | <2.0| 7.9 | 52.5  | 6.8     | 30.5    |
| 8                   | None      | <5.0| 96.0| None  | None    | None    |
|                     | UDP-GlcNAc| <5.0| <5.0| 90.0  | None    | None    |
|                     | UDP-GlcNAc + UDP-Gal | <5.0| <5.0| 61.0  | 29.0    | None    |
|                     | UDP-GlcNAc + UDP-Gal + CMP-NeuAc | <5.0| <5.0| 32.4  | 7.8     | 49.3    |
| 24                  | None      | 6.8 | 93.2| None  | None    | None    |
|                     | UDP-GlcNAc| 7.0 | <3.0| 90.0  | None    | None    |
|                     | UDP-GlcNAc + UDP-Gal | 7.0 | <3.0| 54.2  | 35.8    | None    |
|                     | UDP-GlcNAc + UDP-Gal + CMP-NeuAc | 7.0 | <3.0| 17.3  | 6.5     | 86.2    |

*M, mannose; N, N-acetylglucosamine; Gal, galactose; S, N-acetylneuraminic acid.*

Fig. 4. Fractionation of pronase-digested glycoproteins from control and swainsonine-treated fibroblasts before and after Endo H treatment. The cells were labeled with [3H]mannose for 24 h, and the residue left after extraction of oligosaccharide-lipids with chloroform:methanol:H₂O (10:10:3) was treated with pronase as described under "Experimental Procedures." A-C, controls; D-F, swainsonine-treated cells. The resulting glycopeptides (A and D) were resolved on a Bio-Gel P-4 column (1 x 214 cm, 400 mesh) before (C) and after (E) Endo H treatment. Glycopeptide fractions 101 to 140 of each column were pooled and dried in a Bio-Dryer; the resulting glycopeptide(s) were treated with Endo H and resolved on the same Bio-Gel P-4 column (C and F). The P-4 column was equilibrated with 0.1 M acetic acid, and 0.6-ml fractions were collected at a flow rate of 1.8 ml/h. Standard oligosaccharides were the same as in Fig. 1.
The peak fractions from the control and swainsonine-treated fibroblasts (Fig. 4) were pooled and dried in a Bio-Dryer. The dried oligosaccharides were suspended in water and aliquots containing ~3000 cpm were used for each mannosidase digestion.

| Isolated oligosaccharide | Jack bean mannosidase treatment | Assigned structure |
|--------------------------|---------------------------------|-------------------|
| Oligosaccharide from Fig. 4, C and F | Control (Fig. 4C) | Experimental (Fig. 4F) | % [3H]mannose released |
| Peak 1 | 73.3% (88.9) | 72.3% (88.9) | Man,GlcNAc |
| Peak 2 | 74.9 (87.5) | 75.1 (87.5) | Man,GlcNAc |
| Peak 3 | 74.7 (85.7) | 76.9 (85.7) | Man,GlcNAc |
| Peak 4 | 77.9 (83.3) | 74.3 (83.3) | Man,GlcNAc |
| Peak 5 | 74.2 (80.0) | 75.1 (80.0) | Man,GlcNAc |
| Oligosaccharide from Fig. 4E | | | |
| Peak 1, Fig. 4E | 49.2% (60.0) | NeuAcGalGlcNAcMan,GlcNAc |
| Peak 2, Fig. 4E | 48.8% (60.0) | GalGlcNAcMan,GlcNAc |

* Incubations were carried out at 37 °C for 24 h with jack bean mannosidase as described (11). The theoretical value for releasable [3H]mannose for each oligosaccharide is shown in parentheses.

* Lower levels of [3H]mannose released than theoretically expected is perhaps in part due to some contamination with GlcMan,GlcNAc, which would also be eluted at the position of Peak 1.

* No results are given for control fibroblasts because hybrid oligosaccharides were not observed (see Fig. 4B).

* If the oligosaccharide is incubated with neuraminidase before treatment with jack bean mannosidase and b-D-galactosidase as described under "Experimental Procedures" and applied to a Bio-Gel P-4 column, the product eluted from the column at the position expected of GalGlcNAcMan,GlcNAc. This oligosaccharide as well as the oligosaccharide of Peak 2 (Fig. 4E), when incubated with jack bean a-D-galactosidase and treated with specific glycosidases, resulted in the elution profile observed from control cells (Fig. 4B). The new distinct peaks (Peak 1 and Peak 2, Fig. 4E) were not cleaved by Golgi mannosidase II. However, when sialyl and galactosyl residues were removed by neuraminidase and b-D-galactosidase treatment, the resulting oligosaccharide (GalGlcNAcMan,GlcNAc) becomes an excellent substrate for Golgi mannosidase II.

Table III

| TABLE III | Susceptibility to jack bean α-β-mannosidase of [3H]mannose-labeled processing intermediates from fibroblasts |
|-----------|------------------------------------------------------------------------------------------------------------------|
| Oligosaccharide | Jack bean mannosidase treatment | Experimental (Fig. 4F) | % [3H]mannose released |
| Peak 1 | 73.3% (88.9) | 72.3% (88.9) | Man,GlcNAc |
| Peak 2 | 74.9 (87.5) | 75.1 (87.5) | Man,GlcNAc |
| Peak 3 | 74.7 (85.7) | 76.9 (85.7) | Man,GlcNAc |
| Peak 4 | 77.9 (83.3) | 74.3 (83.3) | Man,GlcNAc |
| Peak 5 | 74.2 (80.0) | 75.1 (80.0) | Man,GlcNAc |

As shown in Table III, the peak fractions from the control and swainsonine-treated fibroblasts (Fig. 4) were pooled and dried in a Bio-Dryer. The dried oligosaccharides were suspended in water and aliquots containing ~3000 cpm were used for each mannosidase digestion.

(only ~5% [3H]mannose released).

The distinctive oligosaccharides from the swainsonine-treated cells (Peaks 1 and 2, Fig. 4E) were characterized. Peak 1 oligosaccharide was identified as sialylated hybrid with the structure NeuAcGalGlcNAc([3H]Man)_5GlcNAc on the basis of its elution from the P-4 column and treatment with specific glycosidases. This oligosaccharide is sensitive to jack bean mannosidase, which cleaved 46% of the 60% theoretically susceptible [3H]mannose (Table III). However, Golgi mannosidase II, which cleaves the exposed α1-3- and α1-6-mannosyl residues from GlcNAcMan,GlcNAc (8, 11), failed to hydrolyze this oligosaccharide, which contains similarly linked mannosyl residues. Treatment of the oligosaccharide with neuraminidase and b-D-galactosidase resulted in the elution of oligosaccharide from a P-4 column at the position expected of GlcNAcMan,GlcNAc (11). This product was sensitive to both jack bean mannosidase and Golgi mannosidase II (Table III). Peak 2, Fig. 4E, was identified as GalGlcNAc([3H]Man)_5GlcNAc, based upon the same type of evidence as given above.

It may be noted that both control and swainsonine-treated cells showed several high mannose oligosaccharides (Fig. 4, C and F), which were identified by their position of elution from the P-4 column and digestion with jack bean mannosidase (Table III). The high mannose oligosaccharides ([3H]Man)_5GlcNAc made up nearly 70% of total radioactivity, and no difference in the total amount of these oligosaccharides was observed between control and swainsonine-treated cells (Fig. 4, C and F).

Table IV

| TABLE IV | Binding of labeled oligosaccharides to serotonin-Sepharose 4B |
|-----------|---------------------------------------------------------------|
| Oligosaccharide | Radioactivity |
| -- | % Unadsorbed / Adsorbed |
| NeuAcGalGlcNAcMan,GlcNAc | 94.5 % (5.0) |
| GalGlcNAc([3H]Man)_5GlcNAc | 93.0 % (7.0) |
| GalGlcNAc([3H]Man)_5GlcNAc | 92.0 % (8.0) |
| NeuAcGalGlcNAc([3H]Man)_5GlcNAc | 93.5 % (6.5) |
| NeuAcGalGlcNAc([3H]Man)_5GlcNAc | 94.5 % (5.5) |
| NeuAcGalGlcNAc([3H]Man)_5GlcNAc | 95.0 % (5.0) |
| NeuAcGalGlcNAc([3H]Man)_5GlcNAc | 96.0 % (4.0) |
| NeuAcGalGlcNAc([3H]Man)_5GlcNAc | 97.0 % (3.0) |
| NeuAcGalGlcNAc([3H]Man)_5GlcNAc | 98.0 % (2.0) |
| NeuAcGalGlcNAc([3H]Man)_5GlcNAc | 99.0 % (1.0) |

* Standard oligosaccharides. See "Experimental Procedures."
Effect of Swainsonine on Glycoprotein Biosynthesis

produced by swainsonine-treated cells is hybrid-type oligosaccharides with neuraminidase and galactosidase treatment. It therefore behaves like high mannose-type oligosaccharides on one branch, it behaved like high mannose-type oligosaccharides in both types of cells were MansGlcNAc and GalGlcNAcMan, and another branch containing terminal al-6-mannosyl residues.

The evidence for the production of hybrid glycoproteins was primarily based on the properties of the oligosaccharides produced from glycoproteins by Endo H treatment. The position of elution of the oligosaccharides from P-4 columns, the responses of the oligosaccharides to digestion with jack bean α-D-mannosidase, and the use of serotonin-Sepharose 4B affinity resin indicated that the principal new species produced by swainsonine-treated cells is hybrid-type oligosaccharide possessing one branch with the structure NeuAcGalGlcNAcMan, and another branch containing terminal α1-3- and α1-6-mannosyl residues.

Although the hybrid oligosaccharide contains 2 terminal mannose residues, it was resistant to Golgi mannoidase II unless sialyl and galactosyl residues were first removed by neuraminidase and β-galactosidase treatment. It therefore appears that a terminal GlcNAc residue on one branch is required for recognition of substrate by the enzyme.

Two additional observations may be noted. First, although the sialylated hybrid possesses the structure of complex oligosaccharide on one branch, it behaved like high mannose-type oligosaccharide in its sensitivity to Endo H, an observation similar to that reported for Rous sarcoma virus glycoproteins (20). Second, the studies demonstrated the usefulness of the affinity resin serotonin-Sepharose 4B (22), which is highly specific for the adsorption of sialic acid-containing oligosaccharide. The sialylated hybrids produced in vitro and in vivo bind to the resin, but the asialo oligosaccharides do not.

Swainsonine has been isolated from Swainsona sp. (29-31) and locoweed (32), plants that induce in livestock a condition resembling the lysosomal storage disease mannosidosis. The causes of the neurological symptoms, the numerous intracellular vacuoles, and the accumulation of mannose-rich oligosaccharides have not been established, although the fact that swainsonine is a potent inhibitor of lysosomal α-D-mannosidase has led to the suggestion that the alkaloid is the specific cause of the mannosidosis (30, 31, 33). However, since the administration of swainsonine to rats induces large increases in lysosomal α-mannosidase of the liver and brain of rats, there is question as to whether swainsonine is in fact primarily responsible for all of the major symptoms of animals ingesting swainsonine-containing plants. Although swainsonine administration to rats raises the level of lysosomal α-mannosidases, it markedly lowers the activity of Golgi mannose-dase II but not of other Golgi enzymes tested. This effect of swainsonine on mannose-dase II in vitro, which is consistent with the in vitro effects of the alkaloid on this enzyme (11), obviously is the cause of the formation of hybrid glycoproteins.

The biological consequences of the production of hybrid glycoproteins in place of complex glycoproteins are difficult to predict at this time. The function of the oligosaccharides of glycoproteins is a subject of considerable contemporary interest, with suggestive evidence on functions deriving in part from the discovery of several carbohydrate-specific receptors on cells (34). The chemical and biological properties of glycoproteins are, in many cases, affected by inhibitors which cause alterations in the structure of the oligosaccharide moieties (35). In a study involving swainsonine, Elbein et al. (28) reported that influenza virus produced in chick embryo fibroblasts in the presence of the alkaloid has normal infectivity in spite of the abnormality of its glycoprotein. Tunica- mycin, on the other hand, prevents the production of infectious virus particles (36). Since swainsonine induces the formation of hybrid oligosaccharides in both human fibroblasts and in the Golgi membrane system described herein, the substance should have wide applicability in investigations of glycoprotein processing, transport, and function. It also seems likely that the pathological effects of swainsonine will in part be explained by its effect on glycoproteins.

Acknowledgments—We are grateful to Kelley Moremen for advice and for essential assistance with the cell culture experiments and to Vera Coleman for excellent technical assistance. We are greatly indebted to Dr. H. F. Broquiot, T. M. Harris, S. C. Hubbard, and P. W. Robbins for substances needed in this study.

Note Added in Proof—After submission of this paper, the presence in P388D mouse macrophage-like cell glycoprotein of hybrid oligosaccharide similar to those reported in the present paper was published (Varki, A., and Kornfeld, S. (1983) J. Biol. Chem. 258, 2808-2818). In addition, swainsonine is reported to induce the formation of hybrid type structures in α-antitrypsin (Gross, V., Tran-Thi, T. A., Vosbeck, K., and Heinrich, P. A. (1983) J. Biol. Chem. 258, 4022-4036) and in vesicular stomatitis virus G protein (Kang, M. S., and Elbein, A. D. (1983) J. Virol. 46, 60-68).

REFERENCES

1. Li, E., Tabas, l., and Kornfeld, S. (1978) J. Biol. Chem. 253, 7762-7770
2. Hubbard, S. C., and Robbins, P. W. (1979) J. Biol. Chem. 254, 4568-4576
3. Chen, W. W., and Lennarz, W. J. (1978) J. Biol. Chem. 253, 5750-5755
4. Spiro, R. G., Spiro, M. J., and Bhyoyroo, V. (1976) J. Biol. Chem. 251, 6420-6425
5. Michael, J. M., and Kornfeld, S. (1980) Arch. Biochem. Biophys. 200, 249-255
6. Hubbard, S. C., and Ivatt, R. J. (1981) Annu. Rev. Biochem. 50, 555-583
7. Tabas, l., and Kornfeld, S. (1979) J. Biol. Chem. 254, 11655-11663
Effect of Swainsonine on Glycoprotein Biosynthesis

11683
8. Tulsiani, D. R. P., Hubbard, S. C., Robbins, P. W., and Touster, O. (1982) J. Biol. Chem. 257, 3660-3668
9. Tabas, I., and Kornfeld, S. (1978) J. Biol. Chem. 253, 7779-7786
10. Harpaz, N., and Schachter, H. (1980) J. Biol. Chem. 255, 4894-4902
11. Tulsiani, D. R. P., Harris, T. M., and Touster, O. (1982) J. Biol. Chem. 257, 7936-7939
12. Tai, T., Yamashita, K., Ito, S., and Kobata, A. (1977) J. Biol. Chem. 252, 6687-6694
13. Yamashita, K., Tsai, C.-Y., and Kobata, A. (1978) J. Biol. Chem. 253, 3862-3869
14. Carver, J. P., Grey, A. A., Winnik, F. M., Hakimi, J., Ceccarini, C., and Atkinson, P. H. (1981) Biochemistry 20, 6600-6606
15. Liang, C.-J., Yamashita, K., Muelenburg, C. G., Shichi, H., and Kobata, A. (1979) J. Biol. Chem. 254, 6414-6418
16. Brenchley, R., and Kornfeld, R. (1980) Arch. Biochem. Biophys. 201, 160-173
17. Anderson, D. R., and Grimes, W. J. (1982) J. Biol. Chem. 257, 14858-14864
18. Ishihara, H., and Tejima, S. (1983) Biochemistry 22, 1194-1201
19. Hunt, L. A., and Wright, S. E. (1983) J. Virol. 45, 233-240
20. Hunt, L. A., and Wright, S. E. (1981) J. Virol. 39, 646-650
21. Schneider, M. J., Ungemach, F. S., Broquist, H. P., and Harris, T. M. (1983) Tetrahedron 39, 29-32
22. Sturgeon, R. J., and Sturgeon, C. M. (1982) Carbohydr. Res. 103, 213-219
23. Leelavathi, D. E., Estes, L. W., Feingold, D. C., and Lombardi, R. (1970) Biochem. Biophys Acta 1178, 124-138
24. Tulsiani, D. R. P., Opheim, D. J., and Touster, O. (1977) J. Biol. Chem. 252, 3227-3233
25. Waechter, C. J., Lucas, J. J., and Lennarz, W. J. (1973) J. Biol. Chem. 248, 7570-7579
26. Anderson, P. M., and Desnick, R. J. (1979) J. Biol. Chem. 254, 11782-11783
27. Elbein, A. D., Solt, R., Dorling, P. R., and Vosbeck, K. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7393-7397
28. Elbein, A. D., Dorling, P. R., Vosbeck, K., and Horisberger, M. (1982) J. Biol. Chem. 257, 1573-1576
29. Dorling, P. R., Huxtable, C. R., and Colegate, S. M. (1980) Biochem. J. 191, 649-651
30. Colegate, S. M., Dorling, P. R., and Huxtable, C. R. (1979) Aust. J. Chem. 32, 2257-2264
31. Dorling, P. R., Huxtable, C. R., and Voegel, P. (1978) Neuropharmacology 17, 285-295
32. Molyneux, R. J., and James, L. F. (1982) Science (Wash. D. C.) 216, 190-191
33. Jolly, R. D., Winchester, B. G., Gehler, J., Dorling, P. R., and Dawson, G. (1981) J. Appl. Biochem. 3, 273-291
34. Ashwell, G., and Harford, J. (1982) Annu. Rev. Biochem. 51, 531-554
35. Schwarz, R. T., and Datema, R. (1982) Adv. Carbohydr. Chem. Biochem. 40, 287-379
36. Schwarz, R. T., Rohrenscheider, J. M., and Schmidt, M. F. G. (1976) J. Virol. 19, 782-791
Swainsonine causes the production of hybrid glycoproteins by human skin fibroblasts and rat liver Golgi preparations.
D R Tulsiani and O Touster

J. Biol. Chem. 1983, 258:7578-7585.

Access the most updated version of this article at http://www.jbc.org/content/258/12/7578

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/12/7578.full.html#ref-list-1