

Review

Glycoprotein Processing and Glycoprotein Processing Inhibitors¹

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ABSTRACT

Considerable evidence is now accumulating from both *in vivo* and *in vitro* studies that the oligosaccharide chains of the plant N-linked glycoproteins undergo modification or processing reactions after the oligosaccharide has been transferred from its lipid-linked oligosaccharide intermediate to the protein. These processing reactions occur in the endoplasmic reticulum and Golgi apparatus of the cell and involve the removal of certain sugars and the addition of other sugars. While the processing reactions appear to be generally similar to those that occur in animal cells, there are some notable differences, such as the addition of a β -linked xylose to many of the plant glycoproteins. It will be interesting to determine the exact sequence of these reactions and how they are regulated in the cell. Recently, some very useful inhibitors have become available that act on the glycosidases that catalyze the removal of glucose and mannose. These inhibitors cause the accumulation of aberrant oligosaccharide chains on the glycoproteins. Such unusual glycoproteins should be valuable tools for studies on the role of oligosaccharide in glycoprotein function.

BIOSYNTHESIS AND PROCESSING OF N-LINKED OLIGOSACCHARIDES

The oligosaccharide chains of the N-linked glycoproteins of eucaryotic cells may have a variety of different kinds of structures ranging from high-mannose types that have only mannose and GlcNAc to highly modified types that, in addition to mannose and GlcNAc, may contain various other sugars such as D-galactose, L-fucose, and sialic acid in animal cells, or D-galactose, L-fucose, and D-xylose in plant cells (20). Although in most cases, the role(s) of the various oligosaccharide chains remains a mystery, a great deal of information is becoming available about the biosynthetic pathway. Furthermore, a number of plant alkaloids and related compounds that inhibit glycoprotein processing are proving to be useful tools to learn more about glycoprotein function.

The initial assembly of the oligosaccharide chains of the N-linked glycoproteins occurs in the ER as a result of a series of lipid-mediated reactions. This pathway involves the sequential addition of the sugars GlcNAc, mannose, and glucose to dolichylphosphate resulting in the formation of a large oligosaccharide linked to dolichol via a pyrophosphoryl bridge, and referred to as $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$ -pyrophosphoryl-dolichol. Many of the lipid-linked oligosaccharide intermediates have been isolated or biosynthesized in various plant systems (reviewed in Ref. 4), and $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$ -PP-dolichol has been demonstrated in sev-

eral plants (12). This oligosaccharide is believed to be the initial precursor for all of the different N-linked oligosaccharides, and is transferred from the lipid carrier to specific asparagine residues that exist in the sequence Asn-X-Ser (Thr), and probably are located on a β -turn of the protein. Glycosylation is generally believed to occur as a co-translation event in the lumen of the endoplasmic reticulum while the polypeptide chain is being synthesized. However, there are some studies that indicate that glycosylation may occur after the synthesis of the protein has been completed (reviewed in Ref. 6). In addition, studies with insects and protozoa have suggested that some lipid-linked oligosaccharides that do not contain glucose (*i.e.* $\text{Man}_{7-9}(\text{GlcNAc})_2$) may still be transferred to protein (15).

Once the oligosaccharide has been transferred to protein, a series of enzymic steps occur that result in the modification of the oligosaccharide chains and give rise to the many-varied structures found in nature (Fig. 1). Thus, in the ER of eucaryotic cells, two different α -glucosidases (referred to as glucosidase I and glucosidase II) remove the three glucoses to give a $\text{Man}_9(\text{GlcNAc})_2$ -protein. These enzymes have been highly purified from animal cells and are quite specific for the glucose containing oligosaccharides (9, 22). However, nothing is known about the regulation of these reactions, *i.e.* how both enzymes distinguish glucose residues on the proteins from those on the lipid-linked saccharides? Is this due to accessibility of oligosaccharides, or to compartmentalization or specificity of the enzyme? Do the glucoses get excised while the protein is still attached to polysomes, or only after polypeptide synthesis is completed? Does the removal of glucose signal the transfer of protein from the ER to the Golgi? Do all N-linked oligosaccharides initially contain glucose?

In animal cells, several other enzymic activities have recently been described that also appear to be localized in the ER (or in the ER-Golgi). The presence of these activities indicates that the processing pathway is more complex than originally believed. One of these enzymes has been found in liver and has endomannosidase activity, *i.e.* this enzyme cleaves a glucosyl α 1,3-mannose disaccharide from the $\text{Glc}_1\text{Man}_9(\text{GlcNAc})_2$ -protein to give a $\text{Man}_8(\text{GlcNAc})_2$ -protein (14). This enzyme could have an important role in the processing since various studies have indicated that glucosidase II removes the first glucose from $\text{Glc}_2\text{Man}_9(\text{GlcNAc})_2$ -protein quite rapidly, but acts on the next glucose very slowly. Perhaps the endomannosidase is really responsible for the removal of this third glucose, or perhaps it plays some key role in processing or targeting? Another enzyme in the ER is the α -mannosidase that removes only one or several of the α 1,2-linked mannoses from the $\text{Man}_9(\text{GlcNAc})_2$ -protein (or perhaps from the $\text{Man}_8(\text{GlcNAc})_2$ -protein) to give Man_8 or $\text{Man}_6(\text{GlcNAc})_2$ -proteins (2). This mannosidase may explain how normal residents of the ER such as hydroxymethylglutaryl-CoA reductase or ribophorin can have high mannose structures with oligosaccharides containing only 6 to 8 mannose residues. At this

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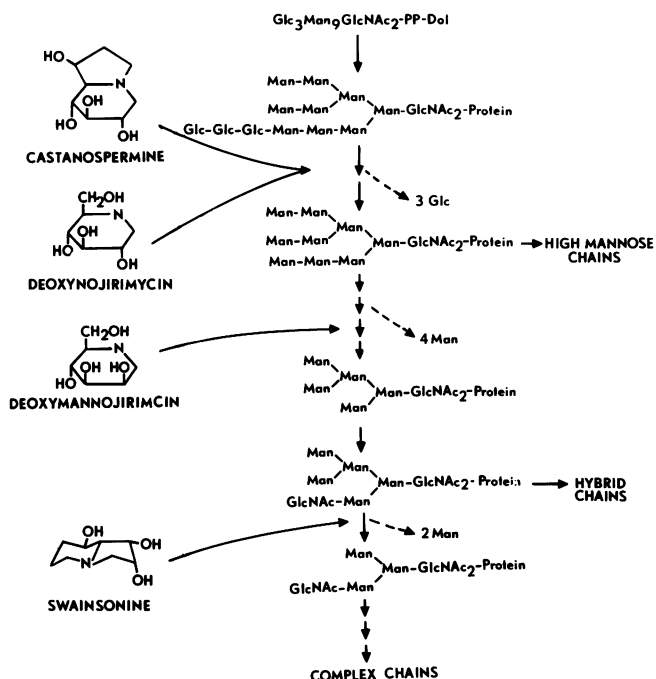


FIG. 1. Site of action of glycoprotein processing inhibitors. The processing pathway of N-linked oligosaccharides involves the removal of some sugars and the addition of other sugars to form the high mannose, hybrid or complex (modified) oligosaccharide chains. The processing inhibitors, shown at the left, act at the specific enzymic steps shown by the arrows. Thus, castanospermine and deoxynojirimycin are inhibitors of glucosidase I (and glucosidase II), deoxymannojirimycin is an inhibitor of mannosidase I and swainsonine is an inhibitor of mannosidase II. Abbreviations are as follows: Glc = glucose; Man = mannose, GlcNAc = N-acetylglucosamine, PP = pyrophosphoryl bridge, Dol = dolichol.

stage, we do not have any evidence for the existence of such enzymes in plants, but glucosidase I and glucosidase II have been isolated and purified from several plant sources.

Following the removal of all of the glucoses, and transfer of the protein from the ER to the Golgi, mannosidase I may remove from 1 to 4 of the α -1,2-linked mannoses to give a Man_n - to $\text{Man}_5(\text{GlcNAc})_2$ -protein. Why some proteins, such as soybean lectin, retain all nine mannoses whereas others are partially or completely susceptible to this mannosidase remains a puzzle. Some studies have attributed this to the accessibility of the oligosaccharide chains to the appropriate enzyme but other regulatory factors may also exist (6, 7). In the event that four mannoses are excised by mannosidase I, the resulting $\text{Man}_5(\text{GlcNAc})_2$ -protein is a substrate for the Golgi GlcNAc transferase I that adds a GlcNAc, from UDP-GlcNAc, to the mannose residue that is linked in α 1,3-linkage to the β -linked mannose. In animal cells, this GlcNAc residue appears to play an important role in directing further reactions of the oligosaccharide chains. Thus, fucosylation or sulfation of N-linked oligosaccharides requires the presence of this GlcNAc, as does the action of the next processing enzyme, mannosidase II. We do not know whether this GlcNAc is necessary for the plant fucosyl transferase or what the substrate specificity of the plant mannosidase II is. However, specificity studies on the Golgi fucosyltransferase of *Phaseolus vulgaris* indicated that all of the oligosaccharides that acted as acceptors of fucose did contain this GlcNAc (11). It will be important to determine what effect the addition of this GlcNAc to the α 3-linked mannose has on further processing in plant systems, in both *in vitro* and *in vivo* experiments. The GlcNAc

transferase I of plant cells was detected in Golgi fractions and showed a strong preference for $\text{Man}_5(\text{GlcNAc})_2$ -Asn as a substrate; $\text{Man}_7(\text{GlcNAc})_2$ -Asn and $\text{Man}_9(\text{GlcNAc})_2$ -Asn were not acceptors of GlcNAc (11).

The mannosidase II of animal cells that removes the two mannose residues linked to the α 6-mannose branch is absolutely dependent on the prior action of GlcNAc transferase I (16, 24). Thus, mannosidase II will not work on $\text{Man}_5(\text{GlcNAc})_2$ -protein, but only on $\text{GlcNAc-Man}_5(\text{GlcNAc})_2$ -protein. This GlcNAc transferase I-dependent α 3(α 6)mannosidase is considered to be the first committed step towards the synthesis of complex or modified oligosaccharides. The highly purified enzyme (16) catalyzes the release of both the α 3- and α 6-linked mannose residues from the $\text{GlcNAc-Man}_5(\text{GlcNAc})_2$ -protein, but it is not known which mannose is cleaved first. Again details regarding the plant enzyme are not yet available.

The product of mannosidase II, *i.e.* $\text{GlcNAc}\beta$ 1,2- $\text{Man}\alpha$ 1,3[$\text{Man}\alpha$ 1,6] $\text{Man}\beta$ GlcNAc-GlcNAc-protein, is probably the substrate for GlcNAc transferase II, but this oligosaccharide can also be acted upon by GlcNAc transferase III (18). While GlcNAc transferase II adds a GlcNAc to the α 6-linked mannose to give $\text{GlcNAc}\beta$ 1,2 $\text{Man}\alpha$ 1,6($\text{GlcNAc}\beta$ 1,2 $\text{Man}\alpha$ -1,3) $\text{Man}\beta$ GlcNAc-GlcNAc-protein, GlcNAc transferase III adds a bisecting GlcNAc to give $\text{GlcNAc}\beta$ 1,2 $\text{Man}\alpha$ 1,3[$\text{GlcNAc}\beta$ 1,4]($\text{Man}\alpha$ 1,6) $\text{Man}\beta$ GlcNAc-GlcNAc-protein. Since GlcNAc transferase III prefers the product of GlcNAc transferase II (*i.e.* $\text{GlcNAc}_2\text{-Man}_3[\text{GlcNAc}]_2$ -protein) by about 8:1 over the $\text{GlcNAc-Man}_3(\text{GlcNAc})_2$ -protein (18), it seems likely that the bisecting GlcNAc is added after the 6-linked GlcNAc, if it is added at all (thus far, no bisecting GlcNAc residues have been reported in plant glycoproteins). Additional evidence that plants may not contain GlcNAc transferase III is the finding that $\text{GlcNAc}_2\text{Man}_3(\text{GlcNAc})_2$ -Asn would not act as an acceptor for an additional GlcNAc by the Golgi fraction of beans. GlcNAc transferase II was purified about 3000-fold from mung beans and was shown to add a GlcNAc in β -1,2 linkage to the $\text{GlcNAc-Man}_3\text{GlcNAc}$. However, $\text{Man}_3\text{-GlcNAc}$, $\text{Man}_5\text{-GlcNAc}$, and $\text{GlcNAc-Man}_5\text{GlcNAc}$ would not act as acceptors for this enzyme (23).

It is also possible that some of these partially modified oligosaccharides may be fucosylated. As mentioned above, *in vitro* studies on the animal fucosyltransferase indicated that the addition of GlcNAc to the 3-linked mannose branch was a necessary signal for this enzyme to add an L-fucose to the innermost GlcNAc residue (13). When swainsonine, an inhibitor of mannosidase II, is fed to cultures of animal cells, these cells accumulate glycoproteins with hybrid types of oligosaccharides which frequently contain fucose (19). Thus, fucosylation may occur just after the action of GlcNAc transferase I, or at some step thereafter. The exact stage at which other sugars, such as galactose, GlcNAc or sialic acid, are added is not entirely clear. In fact, these transferases may not be absolutely specific for a given structure and may be able to add these sugars at several different steps in the pathway (*i.e.* oligosaccharide structures).

One of the unusual and perhaps most interesting aspects of plant N-linked glycoproteins is the presence of a D-xylose attached in β -linkage to the β -linked mannose residue (4, 20). This bisecting xylose may act as some sort of signal for directing further modifications of the oligosaccharide chain or for preventing additional modifications of the oligosaccharide chain as has been suggested for the bisecting GlcNAc of animal cells. The substrate specificity of the xylosyltransferase was recently examined using a Golgi fraction isolated from cotyledons of *P. vulgaris* (11). The xylosyltransferase showed significant activity only with $\text{GlcNAc-Man}_3(\text{GlcNAc})_2$ -Asn and with $\text{GlcNAc}_2\text{-Man}_3(\text{GlcNAc})_2$ -Asn, but was not active on the $\text{GlcNAc-Man}_5(\text{GlcNAc})_2$ -Asn. On the other hand, the plant fucosyltrans-

ferase showed activity with all three oligosaccharide acceptors.

EFFECTS OF GLYCOSIDASE INHIBITORS ON PROCESSING OF OLIGOSACCHARIDES

Some relatively specific inhibitors of the glycosidases that are involved in glycoprotein processing have recently become available (Fig. 1), and these compounds have been very valuable tools to study these glycosidases and their importance in glycoprotein function. Interestingly enough, several of these compounds are indolizidine alkaloids which are found in toxic, wild plants that represent a major hazard to the livestock industry. Thus, swainsonine ([1S,2S,8R,8 α R]-1,2,8-trihydroxyoctahydroindolizidine) is found in *Astragalus* species (locoweed) which grows in the southwestern United States, or in *Swainsona* species which grows in Australia. Animals that eat these plants suffer severe symptoms including skeletal and neurological difficulties and eventual death. Swainsonine is a potent competitive inhibitor of α -mannosidases, such as lysosomal α -mannosidase or jack bean α -mannosidase. With these enzymes, the K_i for inhibition is of the order of 1×10^{-7} M, and it has been suggested that the inhibitory action of this alkaloid results from the structural similarity of its protonated form to the mannosyl cation, since the glycosyl ion intermediate is presumably formed during hydrolysis of natural substrates by glycosidases (6, 7).

In cultured cells incubated in the presence of swainsonine, most of the newly synthesized N-linked glycoproteins have hybrid types of oligosaccharide structures. This is in keeping with the fact that swainsonine inhibits the processing mannosidase II but has no effect on mannosidase I (5, 25). Swainsonine has been used in a number of studies in order to determine whether changes in the structure of the N-linked oligosaccharides affect glycoprotein function (6, 7). In most cases, swainsonine has little effect on the glycoprotein in question which may suggest that a partial complex chain is sufficient for activity, and/or that protein conformation is not altered under these conditions. For example, swainsonine did not impair the synthesis or export of: (a) thyroglobulin in porcine thyroid cells, (b) surfactant glycoprotein A from Type II epithelial cells, (c) H2-DK histocompatibility antigens from macrophages, or (d) von Willebrand protein in epithelial cells. In one report, the secretion of α_1 -antitrypsin from primary rat hepatocytes was not affected by swainsonine, but in another study with human hepatoma cells, swainsonine increased the rate of secretion of transferrin, ceruloplasmin, α_2 -macroglobulin, and α_1 -antitrypsin (reviewed in Ref. 6). The authors suggest that these proteins traverse the Golgi more rapidly than their normal counterparts, but no reason for this alteration in rate was postulated.

Swainsonine also did not affect the insertion or function of the insulin receptor, the epidermal growth factor receptor, or the receptor for asialoglycoproteins. The alkaloid did, however, block the receptor-mediated uptake of mannose-terminated glycoproteins by macrophages. This inhibition appeared to be due to the formation of hybrid chains on the glycoproteins present at the macrophage surface which could then bind to and 'tie-up' the mannose receptors (6). As indicated above, swainsonine also did not prevent the fucosylation (or the sulfation) of N-linked glycoproteins in several animal systems. Fucosylation of glycopeptides and/or glycoproteins in several different plant systems was also not inhibited by swainsonine (3, 10).

However, swainsonine apparently does affect the functions of some glycoproteins. For example, the stimulation of resorptive cells by glucocorticoid probably involves the attachment of osteoclasts and other cells to bone, and this is blocked by swainsonine. The interaction of the parasite, *Trypanosoma cruzi*, with peritoneal macrophages is inhibited or significantly reduced when either the parasite or the host is treated with the drug. B-16-F10

melanoma cells have the ability to colonize the lungs of experimental animals, and this ability is markedly reduced when these animals are given swainsonine. Concanavalin A is a mitogen that can stimulate lymphocytes. This stimulation can be suppressed by an immunosuppressive factor which is found in the serum of mice bearing the tumor, sarcoma 180. The suppression caused by this factor is overcome by swainsonine suggesting that this alkaloid might be a useful tool in immunosuppressive diseases (6, 7). The above studies suggest some important roles for swainsonine and strongly indicate the need for more experimentation with this compound.

Another plant alkaloid that inhibits glycoprotein processing is castanospermine ([1S,6S,7R,8R,8 α R]-1,6,7,8-tetrahydroxyindolizidine). This compound is found in the seeds of the Australian tree, *Castanospermum australe*, also called the Moreton Bay chestnut. Animals that eat these seeds suffer from severe gastrointestinal upset which frequently leads to death. These symptoms are probably related to the fact that castanospermine is a potent inhibitor of α -glucosidases, including intestinal maltase and sucrase (6, 7). As a result, these animals are not able to metabolize starch or sucrose. The site of action of castanospermine in glycoprotein processing is to inhibit glucosidase I (and glucosidase II). Thus, castanospermine prevents the formation of complex oligosaccharides in cell culture, and leads to the formation of high-mannose oligosaccharides that still retain the three glucose residues (17). A related compound in terms of structure and mechanism of action is deoxynojirimycin, a glucose analog in which the oxygen in the pyranose ring is replaced by an NH group. Deoxynojirimycin is produced by certain bacteria of the *Bacillus* species. This compound has also been shown to inhibit the processing glucosidase I (7).

The inability of the cells to remove glucose from their glycoproteins may have dramatic effects on transport, synthesis, and/or secretion of various glycoproteins. For example, in Hep-G2 cells grown in the presence of deoxynojirimycin, the rate of secretion of α_1 -antitrypsin decreased, but only marginal effects were seen on some other glycoproteins, such as ceruloplasmin and the C3 component of complement, or on the 'nonglycoprotein,' albumin (6). Deoxynojirimycin also inhibited α_1 -proteinase inhibitor, cathepsin D, and IgD. It was suggested that the presence of glucose in the oligosaccharide might retard the transport of the protein, and in fact, the inhibited α_1 -antitrypsin was found to accumulate in the endoplasmic reticulum. However, since a number of other glycoproteins with similar types of N-linked oligosaccharide chains are not retarded by deoxynojirimycin, that explanation is not entirely satisfactory. Deoxynojirimycin has also been reported to inhibit the formation of $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2\text{-PP-dolichol}$ in intestinal epithelial cells, and to cause the formation of $\text{Man}_9(\text{GlcNAc})_2\text{-PP-dolichol}$. Furthermore, at high concentrations of deoxynojirimycin, inhibition of glycosylation was observed, and α_1 -antitrypsin forms of lower mol wt were detected. Thus, the decline in the rate of secretion could be a result of a decreased rate of glycosylation. Or, it is possible that for some proteins, the removal of the glucose residues affects the protein structure and causes such proteins to be transported to the Golgi at a more rapid rate. Castanospermine was used with cultured IM-9 lymphocytes to determine the effect of alteration in the carbohydrate structure on the function of the insulin receptor. These cells, treated with this inhibitor, had a 50% reduction in surface insulin receptors as demonstrated by ligand binding, by labeling with Na^{125}I -lactoperoxidase and by cross-linking studies with ^{125}I -insulin. These studies indicated that the removal of glucose from the core oligosaccharide was not necessary for the cleavage of the insulin proreceptor (*i.e.* protein maturation), but the presence of these glucose residues probably does delay the processing of the precursor molecule. Thus, the reduction in the number of surface receptors is prob-

ably the result of an accumulation of the unprocessed receptors in the ER (1).

The effect of swainsonine and deoxynojirimycin on the biosynthesis and processing of phytohemagglutinin was studied in cotyledons of the common bean (3). In the presence of these inhibitors, the oligosaccharide chains of phytohemagglutinin were modified as expected. Thus, deoxynojirimycin gave phytohemagglutinin molecules that were probably glucose-containing high mannose structures, while swainsonine appeared to cause the formation of hybrid structures. Normally, the further processing of this protein involves the addition of terminal GlcNAc residues to the oligosaccharide chains. These GlcNAc units are added in the Golgi apparatus and the GlcNAc residues are removed when the phytohemagglutinin is transported to the protein bodies (4). In the presence of deoxynojirimycin, the addition of GlcNAc was blocked, but attachment of GlcNAc still occurred in the presence of swainsonine. These results are not unexpected since swainsonine inhibits at the step after the addition of this GlcNAc (*i.e.* mannosidase II), whereas deoxynojirimycin inhibits at the first step. Interestingly enough, these inhibitors had no effect on the transport of the phytohemagglutinin from its site of synthesis in the ER-Golgi to its ultimate location in the protein bodies (3).

Based on the fact that deoxynojirimycin inhibits glycoprotein processing at the glucosidase I stage, the mannose analog of this compound, named deoxymannojirimycin, was synthesized chemically (8). This compound did not inhibit lysosomal or jack bean α -mannosidase, nor did it inhibit mannosidase II. It did, however, inhibit the Golgi mannosidase IA/B, and it caused the accumulation of high mannose oligosaccharides of the Man_{8-9} -(GlcNAc)₂ structure in cultured cells. Deoxymannojirimycin did not inhibit the secretion of IgD or IgM (whereas deoxynojirimycin did) in cultured cells, nor did it have any effect on the appearance of the G protein of vesicular stomatitis virus, the hemagglutinin of influenza virus, or the HLA-A, B and C antigens.

In one study, deoxymannojirimycin was used as a tool to study the recycling of membrane glycoproteins through the Golgi regions that contained mannosidase I. Membrane glycoproteins were synthesized and labeled in the presence of deoxymannojirimycin, causing the proteins to have Man_{8-9} -(GlcNAc)₂ structures. The media was then replaced with fresh media to remove the label and the inhibitor, and the change in the structure of the oligosaccharide chains was followed with time. The data indicated that the transferrin receptor and other membrane glycoproteins were transported to the mannosidase I compartment during endocytosis, since a portion of this receptor was acted upon by the mannosidase(s). These studies indicated that some proteins and/or receptors may be reprocessed during endocytosis (21).

Deoxymannojirimycin was also used to examine the role of the ER α -mannosidase in the processing of hydroxymethylglutaryl-CoA reductase, the rate limiting and control enzyme in cholesterol biosynthesis. This enzyme is a glycoprotein that is located in the ER of many animal cells, such as UT-1, a mutant cell line that is resistant to compactin. Under normal conditions with this mutant, the predominant oligosaccharides that are present on the reductase are single isomers of Man_6 -(GlcNAc)₂ and Man_8 -(GlcNAc)₂. However, in the presence of deoxymannojirimycin, the Man_8 -(GlcNAc)₂ accumulated, indicating that the ER α -mannosidase was responsible for the initial mannose processing (2). However, not all hepatocyte glycoproteins were found to be substrates for this ER α -mannosidase. No studies have been reported using deoxymannojirimycin in plant systems.

The three inhibitors described above, *i.e.* castanospermine (and deoxynojirimycin), deoxymannojirimycin, and swainsonine allow one to inhibit glycoprotein processing at three different steps

(Fig. 1). Inhibition at the glucosidase I stage results in the formation of glycoproteins having glucose-containing high mannose structures; inhibition at the mannosidase I step gives glycoproteins with Man_{8-9} -(GlcNAc)₂ oligosaccharides; inhibition at the mannosidase II step produces glycoproteins with hybrid structures. Thus, for any glycoprotein that can be synthesized in cell culture in the presence of these inhibitors, one can modify the oligosaccharide structure and determine how these modifications affect function. A number of studies have been done with these various inhibitors on many different secretory and membrane glycoproteins, but the results seem to depend on the system being used. That is, in some cases modification of the carbohydrate structure may have pronounced effects on the targeting or function of the glycoprotein in question. In other cases, alterations from complex structures to high mannose chains had little effect on targeting, secretion or function. Thus, the significance of the carbohydrate in any of these systems must depend on the structure and/or the conformation of the polypeptide chain itself. As more studies are done on these systems, and as more inhibitors become available that act at other steps in the pathway, the information should meld into a better understanding of why proteins contain covalently bound carbohydrate.

LITERATURE CITED

1. ARAKAKI RF, JA HEDO, E COLLIER, P GORDEN 1987 Effects of castanospermine and l-deoxynojirimycin on insulin receptor biogenesis. Evidence for a role of glucose removal from core oligosaccharide. *J Biol Chem* 262: 11886-11892
2. BISCHOFF J, L LISCUM, R KORNFELD 1986 The use of l-deoxynojirimycin to evaluate the role of various α -mannosidases in oligosaccharide processing in intact cells. *J Biol Chem* 261: 4766-4774
3. CHRISPPEELS MJ, A VITALE 1985 Abnormal processing of the modified oligosaccharide side chains of phytohemagglutinin in the presence of swainsonine and deoxynojirimycin. *Plant Physiol* 78: 704-709
4. ELBEIN AD 1979 The role of lipid-linked saccharides in the biosynthesis of glycoproteins. *Annu Rev Plant Physiol* 30: 239-272
5. ELBEIN AD, P DORLING, R SOLF, K VOSBECK 1981 Swainsonine: an inhibitor of glycoprotein processing. *Proc Natl Acad Sci USA* 78: 7393-7397
6. ELBEIN AD 1987 Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. *Annu Rev Biochem* 56: 497-534
7. FUHRMANN U, E BAUSE, H PLOEGH 1985 Inhibitors of oligosaccharide processing. *Biochim Biophys Acta* 825: 95-110
8. HETTKAMP H, E BAUSE, G LEGLER 1982 Novel mannosidase inhibitor blocking conversion of high mannose to complex oligosaccharides. *Biosci Rep* 2: 899-906
9. HETTKAMP H, G LEGLER, E BAUSE 1984 Purification by affinity chromatography of glucosidase I, an endoplasmic reticulum hydrolase involved in the processing of asparagine-linked oligosaccharides. *Eur J Biochem* 142: 85-90
10. HORI H, GP KAUSHAL, AD ELBEIN 1985 Fucosylation of membrane proteins in soybean culture cells. Effects of tunicamycin and swainsonine. *Plant Physiol* 77: 687-694
11. JOHNSON KD, MJ CHRISPPEELS 1987 Substrate specificities of N-acetylglucosaminyl-, fucosyl- and xylosyltransferases that modify glycoproteins in the Golgi apparatus of bean cotyledons. *Plant Physiol* 84: 1301-1308
12. LEHLE L, W TANNER 1983 Polyphenol-linked sugars and glycoprotein synthesis in plants. *Biochem Soc Trans* 11: 568-574
13. LONGMORE GD, H SCHACHTER 1982 Product-identification and substrate specificity studies of the GDP-fucose:2-acetamido-2-deoxy- β -D-glucoside (Fuc \rightarrow Asn linked GlcNAc) 6- α -L-fucosyltransferase in a Golgi rich fraction from porcine liver. *Carbohydr Res* 100: 365-392
14. LUBAS WA, RG SPIRO 1987 Golgi endo- α -D-mannosidase from rat liver, a novel N-linked carbohydrate processing enzyme. *J Biol Chem* 262: 3775-3781
15. MENDELZON DH, AJ PARODI 1986 N-linked high mannose type oligosaccharides in the protozoan *Criethidia fasciculata* and *Criethidia hamosa* contain galactofuranose residues. *J Biol Chem* 261: 2129-2133
16. MOREMAN KW, O TOUSTER 1985 Biosynthesis and modification of Golgi mannosidase II in hela and 3T3 cells. *J Biol Chem* 260: 6654-6662
17. PAN YT, H HORI, R SAUL, BA SANFORD, RJ MOLYNEUX, AD ELBEIN 1983 Castanospermine inhibits the processing of the oligosaccharide portion of the influenza viral glycoprotein. *Biochemistry* 22: 3975-3984
18. SCHACHTER H, S NARASIMHAN, P GLEESON, G VELLA 1983 Control of branching during the biosynthesis of asparagine-linked oligosaccharides. *Can J Biochem Cell Biol* 61: 1049-1066
19. SCHWARTZ P, AD ELBEIN 1985 The effect of glycoprotein processing inhibitors on fucosylation of glycoproteins. *J Biol Chem* 260: 1083-1089
20. SHARON N, H LIS 1979 Comparative biochemistry of plant glycoproteins. *Biochem*

- Soc Trans 7: 783-789
21. SNIDER MD, OC ROGERS 1986 Membrane traffic in animal cells: cellular glycoproteins return to the site of Golgi mannosidase I. J Cell Biol 103: 265-275
 22. STROUS GJ, P VANKERKHOF, R BROK, J ROTH, D BRADA 1987 Glucosidase II, a protein of the endoplasmic reticulum with high mannose oligosaccharide chains and a rapid turnover. J Biol Chem 262: 3620-3625
 23. SZUMILO T, GP KAUSHAL, AD ELBEIN 1987 Purification and properties of the glycoprotein processing *N*-acetylglucosaminyltransferase II. Biochemistry 26: 5498-5505
 24. TABAS I, S KORNFELD 1978 The synthesis of complex-type oligosaccharides. III. Identification of an α -D-mannosidase activity involved in a late stage of processing of complex type oligosaccharides. J Biol Chem 253: 7779-7786
 25. TULSIANI DPR, TM HARRIS, O TOUSTER 1982 Swainsonine inhibits the biosynthesis of complex glycoproteins by inhibition of Golgi mannosidase II. J Biol Chem 257: 7936-7939