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Effect of Inhibition of Glycosylation on the Appearance of a 60 kD Membrane Glycopolypeptide in Endomembrane Fractions of Soybean Root

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ABSTRACT

Endomembrane (endoplasmic reticulum, Golgi apparatus, plasma membrane) proteins of soybean (*Glycine max*) root cells are highly glycosylated. We investigated whether N-linked oligosaccharide moieties are essential for the correct intracellular transport of plant endomembrane glycoproteins. Excised roots were incubated with tunicamycin, to block cotranslational glycosylation of proteins, and dual labeled with [^{3}H]glucosamine and [^{35}S] (methionine, cysteine). In the presence of tunicamycin, the incorporation of glucosamine into membrane proteins was inhibited by 60 to 90% while amino acid incorporation was only slightly affected. Autoradiograms of two-dimensionally separated polypeptides from each endomembrane fraction revealed the presence of at least one new polypeptide in tunicamycin-treated tissue. The new polypeptide was of the same isoelectric point but lower molecular weight than a preexisting polypeptide. The new polypeptide was unreactive to concanavalin A, as opposed to the preexisting polypeptide, suggesting the absence of the glycan portion. Trifluoromethanesulfonic acid and N-glycanase were used to cleave the carbohydrate from the preexisting concanavalin A binding polypeptide. In each case a deglycosylated polypeptide of the same isoelectric point and molecular weight as the new polypeptide from tunicamycin-treated tissue resulted. Since the absence of carbohydrate from the new endomembrane polypeptide did not prevent its appearance on autoradiograms of Golgi and plasma membrane, intracellular transport and intercalation of newly synthesized glycoproteins into plant cell membranes may not require the presence of polysaccharide moieties.

Glycoproteins are localized in the cytosol, components of membranes, and secreted from the cell, yet the roles that the carbohydrate moieties play in the physiology of the cell are poorly understood. Research, primarily on animal systems, suggests that the carbohydrate portion of glycoproteins may function in: maintaining protein conformation, controlling proteolytic activity, mediating recognition phenomena, regulating development, and sorting intracellular and secretory proteins (reviewed in Ref. 24). Many membrane glycoproteins are classified as N-linked with the carbohydrate linked through the amide nitrogen of asparagine (18). The carbohydrate core sequence of $Glc_3Man_9GlcNAc_2$ of an N-linked glycoprotein is donated from a hydrophobic lipid intermediate to the carboxylamido side chain of specific asparagine residues of the sequence Asn-X-Ser/Thr in the growing polypeptide chain. This occurs as a cotranslational event on the rough endoplasmic reticulum (15). Inhibition of specific steps in the pathway of protein glycosylation is an effective strategy in elucidating the function of the glycan moiety of glycoproteins (7). One of the most specific inhibitors is TM,[1] a nucleoside antibiotic produced by *Streptomyces lysosuperificus*, which blocks the first step in the formation of the lipid-linked saccharide core, *i.e.* the transfer of GlcNAc-1-P from UDP-GlcNAc to dolichyl-P to produce GlcNAc-PP-dolichyl (UDP-GlcNAc:dolichyl-phosphate GlcNAc-1-P transferase) (30). In cultured soybean cells TM completely blocked the incorporation of [^{3}H]mannose into lipid-linked oligosaccharides (14).

TM has been reported to partially inhibit glycosylation in excised *Phaseolus* cotyledons but the assembly, transport to protein bodies, and biological activity of nonglycosylated phytohemagglutinin were not affected (1). We have previously demonstrated that the endomembranes (ER, GA, PM) of soybean root tissue contain numerous glycosylated polypeptides (20). In the present report, we have investigated whether the saccharide moiety of a 60 kD soybean PM glycoprotein is required for its intracellular transport through the membrane biosynthetic pathway. Root tissue was treated with TM and incubated with radiolabeled metabolites to investigate the effect of TM on the synthesis and glycosylation of the endomembrane polypeptide and to determine if 'nonglycosylated glycoproteins' are transported to the GA and PM.

MATERIALS AND METHODS

Plant Tissue

Soybean seeds (*Glycine max* [L.] Merr. cult. Wells II) were germinated in darkness in moist vermiculite at 29°C. Three-d old seedlings were gently removed from the vermiculite and rinsed with deionized H_2O, and 1.5 cm apical root segments were excised and placed in 1 mM K^+-phosphate, 0.5 mM $CaCl_2$ (pH 6.0) at 28°C. Root segments (3.8 g) were incubated 4 h to allow for recovery from excision injury (11).

Tunicamycin Treatment

TM (Sigma; St. Louis, MO) was prepared as a 5 mg/mL stock solution in 10 mM NaOH by heating at 37°C for 3 min. TM was added at a final concentration of 0.1 mg/mL in 30 mL incubation buffer (10 mM K^+-phosphate, 0.5 mM $CaCl_2$, pH 8.0) at 28°C. After a 3 h pretreatment with TM a dual

[1] Abbreviations: TM, tunicamycin; GA, Golgi apparatus; PM, plasma membrane; con A, concanavalin A; TFMSA, trifluoromethanesulfonic acid; BHT, butylated hydroxytoluene; PVPP, polyvinylpolypyrrolidone.

labeling experiment was initiated by adding to the root segments 1.0 mCi of Tran^{35}S-label (about 80% methionine, 15% cysteine; 1104 Ci/mmol), and 125 μCi D-[6-^{3}H]glucosamine HCl (40 Ci/mmol); (ICN Radiochemicals; Irvine, CA). After labeling for 1.5 h the roots were washed for 2 min with ice-cold 1 mM K^+-phosphate, 0.5 mM $CaCl_2$ (pH 6.0) containing 1 mM each nonradioactive methionine, cysteine, and glucosamine.

Isolation of Membrane Fractions

Root tissue was homogenized with 10 mL grinding medium (0.3 M sucrose, 50 mM Tris-Hepes (pH 7.2), 2 mM $MgCl_2$, 2.5 mM DTT, 0.4% [w/v] BSA, 100 μg/mL BHT, 5% [w/tissue w] PVPP) in a mortar and pestle and filtered through Miracloth. Endomembrane-enriched fractions were isolated as previously described (20). After centrifuging 15 min at 13,000g, the supernatant was underlaid with 2.5 mL 34% (w/w) and 2 mL 45% Mg^{2+}-buffered sucrose (1 mM Tris-Hepes [pH 7.2], 1 mM DTT, 10 μg/mL BHT). Gradients were centrifuged for 2.25 h at 80,000g in a SW 27.1 rotor (Beckman; Palo Alto, CA). Material collected at the 34% interface was diluted with 1 volume of 1 mM Tris-Hepes (pH 7.2), 1 mM DTT, 10 μg/mL BHT, 2 mM $MgCl_2$, and underlaid with 7 mL 25% and 6.5 mL 30% Mg^{2+}-buffered sucrose to form one gradient. Material collected at the 34/45% interface was diluted with 2 volumes of 5 mM EDTA, 1 mM Tris-Hepes (pH 7.2), 1 mM DTT, 10 μg/mL BHT to strip ribosomes from rough ER (27, 28) and underlaid with 3 mL 20%, 2.5 mL 30%, 3.5 mL 34%, and 2.5 mL 45% buffered EDTA-sucrose (1 mM Tris-Hepes [pH 7.2], 1 mM EDTA, 1 mM DTT, 10 μg/mL BHT) to form a second gradient. Membrane fractions were recovered at interfaces (GA 25/30%, first gradient; ER 20/30% and PM 34/45%, second gradient) with a bent Pasteur pipet, diluted at least 1:1 with 0.2 mM KCl, 1 mM Tris-Hepes [pH 7.2], 1 mM DTT, 10 μg/mL BHT, and pelleted at 200,000g for 30 min in a Beckman SW 60 rotor. Pellets were resuspended in 10% (w/w) buffered sucrose containing 0.8 mM phenylmethylsulfonyl fluoride (Sigma). All steps were performed at 4°C. Endomembrane fractions prepared by this procedure were relatively free of cross-contamination as determined by marker enzyme analyses (20).

Determination of Radioactivity

Incorporation of radiolabeled precursors was modified slightly from the procedure of Chrispeels (3). Briefly, resuspended membrane pellets were diluted and a 10 μL aliquot was mixed with 10 μL of 10% (w/v) TCA, brought to 3 mL with 5% TCA and incubated at room temperature for 15 min. The protein was collected on glass fiber filters (Whatman GF/C), washed with 5% TCA, dried, and the radioactivity determined in a nonaqueous liquid scintillation cocktail (Beckman Ready-Solv™ NA). The ^{3}H cpm data were corrected for the contribution of ^{35}S-derived cpm in the dual label experiment.

Two-Dimensional IEF-SDS-PAGE Separation of Membrane Polypeptides and Electroelution of Glycopolypeptide 6

The protein concentration of the membrane fractions was estimated in the presence of Triton X-100 as described (10); crystalline bovine serum albumin was the standard. An equal amount of protein (35 μg) from each fraction was diluted to 90 μL containing 53 μg SDS, 15 mM DTT, and heated at 70°C for 20 min. After cooling to room temperature, urea (80 mg), Nonidet P-40 (to 4% v/v), and ampholytes (LKB 3-10, to 2% w/v) were added. The two-dimensional electrophoretic separation was as previously described (2). Two-dimensionally separated ER glycopolypeptide 6 was electroeluted using a Bio-Rad apparatus based on the procedure of Hunkapiller *et al.* (16). Briefly, gels were stained 8 min in 0.3% (w/v) Coomassie blue R-250 in acetic acid:isopropanol:H_2O (10:30:60, v/v) and destained 1 h in acetic acid:isopropanol:H_2O. The lightly stained spot was excised, washed with H_2O for 1.5 h, and stored at −20°C. Pooled gel pieces were eluted with 0.1% SDS in 0.05 M NH_4HCO_3 and dialyzed against 0.007% SDS in 0.05 M NH_4HCO_3. The eluted glycopolypeptide was stored at −20°C until further analysis.

Electrophoretic Transfer of Polypeptides to Nitrocellulose

Polypeptides were transferred to nitrocellulose paper (0.45 μm; Bio-Rad) in a transblot cell (Bio-Rad) according to manufacturer's instructions (in 25 mM Tris, 192 mM glycine, 20% v/v methanol, 0.2% w/v SDS) at 135 mA overnight followed by 1 h at 295 mA. The protein blots were briefly stained with 0.1% (w/v) amido black in ethanol:acetic acid:H_2O (45:10:45, v/v), destained in H_2O, and photographed. Autoradiograms were obtained by exposing Kodak X-AR film to the nitrocellulose blots at −70°C.

Glycoprotein Visualization

Con A binding to immobilized glycopolypeptides was visualized with peroxidase and 4-chloro-naphthol (9, 12, 17). The amido black-stained, photographed and completely destained (ethanol:H_2O [40:60, v/v]) nitrocellulose blots were briefly washed in TBSCT (20 mM Tris (pH 7.4), 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 0.05% Tween 20) and then treated with 50 μg/mL con A (Sigma) in TBSCT for 30 min, washed with four changes in TBSCT over 20 min, reacted with 50 μg/mL peroxidase (type II, Sigma) in TBSCT for 30 min, washed, and reacted with freshly prepared developer (60 mg 40-chloro-napthol [Bio-Rad] in 20 mL cold methanol mixed with 60 μL H_2O_2 in 100 mL 20 mM Tris (pH 7.4), 0.5 M NaCl) for 4 min. The nitrocellulose sheets were washed extensively with H_2O, blotted dry, and photographed. The specificity of con A for glucosyl and mannosyl residues was tested by including the competing ligand, methyl-α-D mannoside, in buffers used for con A binding and washing. This ligand completely inhibited con A binding.

Chemical Deglycosylation

The purified membrane glycopolypeptide 6 was deglycosylated as described by Paaren *et al.* (25). The protein was precipitated with chloroform:methanol:H_2O as described by Wessel and Flugge (32), dried, and dissolved in urea buffer (8 M urea, 2% v/v NP-40, 2% v/v ampholytes) for two-dimensional SDS-PAGE.

Enzymic Deglycosylation

N-Glycanase (Genzyme Corp.) was used to enzymically deglycosylate the eluted ER polypeptide 6. The procedure was similar to that described by Hirani *et al.* (13). The eluted polypeptide was concentrated to near dryness with a stream of N_2 and 2.8 μL 10% v/v NP-40, 10 μL H_2O, and ± 1 μL *N*-glycanase (0.28 unit) were added. The samples were incubated for 20 h at 37°C and prepared for two-dimensional electrophoresis by the addition of 120 μL urea buffer.

RESULTS

The role that saccharide moieties play in targeting newly synthesized membrane glycoproteins to their ultimate intracellular destination was examined. The extent of inhibition of glycosylation and synthesis of endomembrane polypeptides by TM was determined quantitatively by dual labeling with [^{3}H]glucosamine and [^{35}S](methionine, cysteine) and qualitatively by comparing the con A-binding activities of endomembrane polypeptides isolated from control and treated tissue. Con A is a lectin frequently employed to identify glucosyl and/or mannosyl residues of glycoproteins.

The effect of TM on saccharide and amino acid incorporation into soybean root endomembranes is shown in Table I. TM was a potent inhibitor of [^{3}H]glucosamine incorporation into TCA-insoluble proteins. TM was tested for its effect on the uptake of [^{3}H]glucosamine by the root segments to ensure that reduced incorporation was not a result of the inhibition of uptake. While the inhibitor did reduce uptake by 20% (data not shown) this alone was not adequate to account for the observed reduction in incorporation. The greatest inhibition of [^{3}H]glucosamine incorporation into membrane proteins occurred in the ER, the GA was slightly less inhibited, and the PM was the least affected. In contrast, the TM treatment stimulated the incorporation of labeled methionine and/or cysteine in the ER and GA and was only slightly inhibitory in the PM.

Membrane vesicles, isolated as previously described, yielded solubilized protein complements characteristic of each membrane class (20), ruling out significant cross-contamination of membrane fractions. Polypeptides were solubilized from an equal protein amount of each fraction and separated by IEF-SDS-PAGE (Fig. 1).

The amido black stained two-dimensional polypeptide patterns of each endomembrane fraction obtained from TM-treated roots were generally similar to the corresponding fraction from control roots; however, there were slight differences in the relative amounts of several minor polypeptides (Fig. 1; *cf.* A and B, C and D, E and F). In contrast, autoradiograms of the blots shown in Figure 1 revealed apparent increased radioactivity in several membrane polypeptides as a result of the TM treatment (Fig. 2, B, D, and F; polypeptides number 1 (pI 5.2, M_r 76,000); 2 (pI 5.0, M_r 69,000) in each fraction and ER polypeptides 3, 4, 5) and a decrease in the specific activity of ER polypeptide 6 (Fig 2B). The images formed on the x-ray film (Figs. 1B, 2B, 3B) most likely represent amino acids rather than saccharides since the levels of incorporation of the relatively energetic ^{35}S label were 40 to 50 times that of the weakly energetic ^{3}H label (Table I).

A new polypeptide appeared on autoradiograms from endomembranes isolated from TM-treated root tissue (Fig. 2, B, D, and F; arrow 7, (pI 4.6, M_r 55,000)). This newly synthesized polypeptide appeared in each endomembrane fraction just below the position of polypeptide number 6 (pI 4.6, M_r 60,000) (*cf.* Figs. 1 and 2). Intact soybean seedlings treated with TM for 15 h accumulated a sufficient amount of

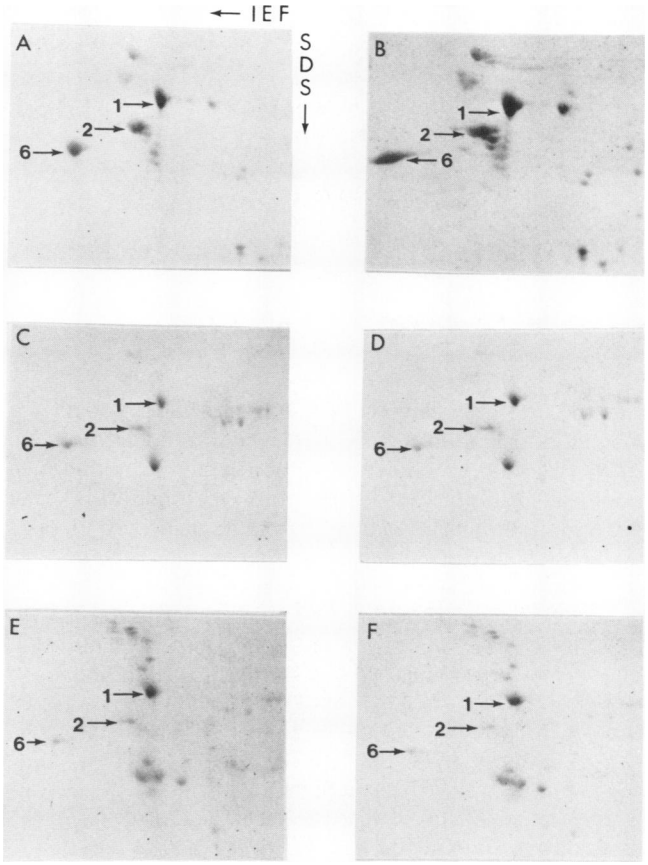

Figure 1. Two-dimensional electrophoretograms of polypeptides from endomembrane fractions of soybean roots. Membranes were isolated from control tissue (A, C, E), and tissue was treated 4.5 h with 0.1 mg/mL tunicamycin (B, D, F). ER-Enriched fraction A, B; GA-enriched fraction C, D; PM-enriched fraction E, F. Each IEF gel received protein solubilized from 35 μg of a membrane fraction. A 10% polyacrylamide gel was used for the second dimension. Polypeptides migrating between pI 4 to 6 and 105 to 42 kD are shown except for B where the pI range was 4.5 to 6. Staining of the nitrocellulose blots was with amido black.

Table I. *Dual-Labeling Study on Effect of TM (0.1 mg/mL) on Incorporation of Radiolabeled Precursors by 1.5 cm Apical Root Segments*

Values in parentheses represent percent of control.

Fraction	Control	[^{3}H]GlcN	[^{35}S](Met,Cys)
		cpm/μg	
ER	Control	970	3890
	TM	102 (10)	5374 (138)
GA	Control	712	3490
	TM	130 (18)	4550 (130)
PM	Control	1380	5430
	TM	550 (40)	4940 (91)

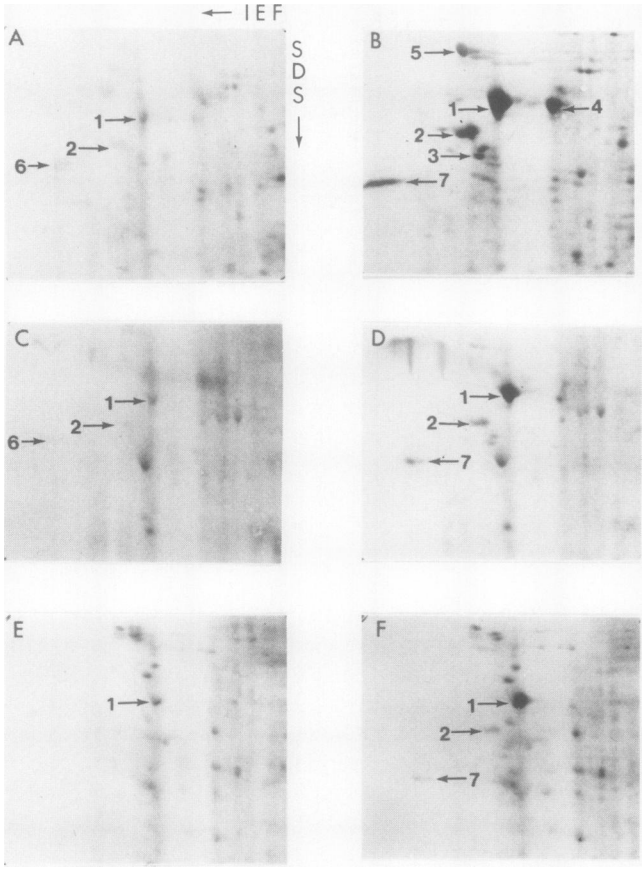

Figure 2. Autoradiograms of the blots shown in Figure 1. Root tissue was dual labeled with [^{35}S](Met, Cys) and [^{3}H]GlcN for 1.5 h following a 3 h pretreatment ± 0.1 mg/mL tunicamycin. Legend as for Figure 1.

polypeptide No. 7 to be detected by amido black staining (Fig. 3B) yet this polypeptide was unable to bind con A (Fig. 3D) in contrast to polypeptide No. 6 (Fig. 3D).

The results suggest that polypeptide 7 (Fig. 2, B, D, and F) represents a form of polypeptide 6 that was synthesized and intracellularly transported to the GA and PM. One approach to test that hypothesis was to demonstrate that removal of the carbohydrate from glycopolypeptide 6 yielded a polypeptide of electrophoretic mobility identical to polypeptide 7. Two methods of glycoprotein deglycosylation (chemical and enzymic) were employed to cleave the saccharide moiety of glycopolypeptide 6 following electroelution from two-dimensional electrophoretograms of ER. Figure 4 shows that the polypeptide produced by either method was approximately 5000 D less than the native polypeptide in agreement with the difference in mol wt seen in Figures 1 and 2 (4.5 h TM treatment) and Fig. 3B (15 h TM treatment). Two polypeptide spots of the same mol wt but differing in pI appeared after TFMSA treatment (Fig. 4, spots B, C). One of these polypeptides (Fig. 4, spot C) was of the same pI (4.6) as control glycopolypeptide 6 (Fig. 4, spot A), while the second polypeptide (Fig. 4, spot B) was more acidic (pI 4.25). Only a single polypeptide resulted from the enzymic deglycosylation (Fig. 4, spot C). Con A-peroxidase staining revealed that the eluted glycopolypeptide 6 bound con A in contrast to the polypep-

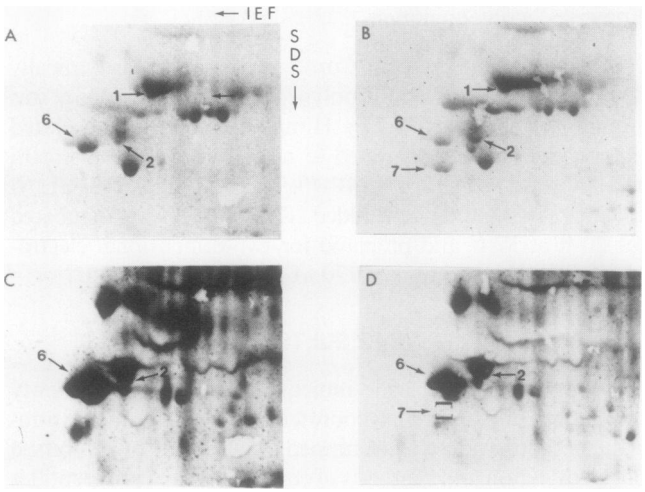

Figure 3. Two-dimensional electrophoretograms of membrane polypeptides isolated from root tissue of intact soybean seedlings. Seedlings were incubated in the absence of tunicamycin (A, C) and in the presence of 0.1 mg/mL tunicamycin for 15 h. Each IEF gel received protein solubilized from 160 μg of a GA-enriched membrane fraction. The nitrocellulose blots were stained with amido black (A, B); destained and probed with con A-peroxidase (C, D). A 10% polyacrylamide gel was used for the second dimension. ER and PM samples also showed a lack of con A binding by polypeptide 7 (position indicated by brackets in D) data not shown.

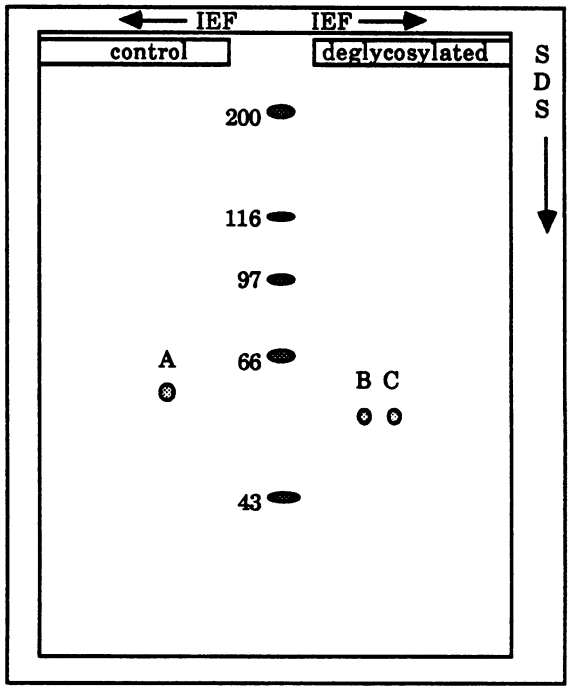

Figure 4. Tracing of a two-dimensional separation of glycopolypeptide 6, eluted from 2-D gels of ER, before and after a deglycosylation treatment. Samples were loaded on the anodic end of the IEF gels. A 7.5% polyacrylamide gel was used for the second dimension. Chemcial deglycosylation produced spots B, C and enzymic deglycosylation produced only spot C. Mol wt standards ($\times 10^{-3}$) were run in the center of the slab gel. Staining was with Coomassie blue.

tides produced by either the chemical or the enzymic treatments (data not shown).

DISCUSSION

The results demonstrate that at least one integral membrane glycopolypeptide of soybean root endomembranes appeared in the GA and PM in a nonglycosylated form following TM treatment.

TM was a very effective inhibitor of [^{3}H]glucosamine incorporation but had little effect on amino acid incorporation into nascent proteins (Table I). The greater inhibition of saccharide incorporation by TM in the ER fraction is in agreement with the tenet that this organelle is the site of membrane-protein synthesis and cotranslational glycosylation (15). In addition to N-linked glycosylation, proteins can be further glycosylated by saccharide addition to the hydroxyl of serine or threonine residues. This represents a posttranslational modification of proteins, termed *O*-glycosylation, that occurs in the GA and, in contrast to N-linked glycosylation, is insensitive to TM (21). The higher incorporation from [^{3}H] glucosamine in the GA and PM relative to the ER (Table I) may represent *O*-glycosylation of plant membrane proteins. Intact lymphocytes have been shown to contain *O*-linked *N*-acetylglucosamine residues on the cell surface (31).

TM is produced by *Streptomyces lysosuperificus* as a mixture of at least 10 major homologues differing in their fatty acid side chains, some of which are inhibitory to protein biosynthesis (5). Therefore, it is important to determine the effect on TM on amino acid incorporation in any study employing this antibiotic. Surprisingly, TM stimulated amino acid incorporation into endomembrane polypeptides in soybean root tissue for both the ER and GA fractions and was only slightly inhibitory in the PM (Table I). The enhanced amino acid incorporation in the presence of TM was also apparent for several membrane polypeptides resolved by two-dimensional electrophoresis (Fig. 2). Whether the stimulation observed was due to the impairment in some yet unknown feedback regulation normally imposed by cotranslational glycosylation or the result of a more general stress response cannot be determined from the present data.

TM has been used in investigations into the function of the glycan moiety of glycoproteins (8). For example, glycosylation may be required for proteins to assume the correct conformation for secretion since TM not only blocked invertase and acid phosphatase glycosylation but also prevented their secretion in yeast (24). Carbohydrate residues are putatively required for targeting delivery to specific organelles, since in the presence of TM, lysosomal enzymes of chick embryo fibroblasts were misdirected and secreted (25). Phytohemagglutinin, a plant storage protein, is synthesized and cotranslationally glycosylated on rough ER then further modified by the addition of fucose in the GA (3). However, in the presence of TM, the nonglycosylated protein was transported to its destination, the protein body (1). In the present study, polypeptide No. 7 was clearly shown to appear in both the GA and PM in a nonglycosylated state. As shown in Figure 2, the radiolabeled polypeptide patterns were similar for both control and TM treatments except for the appearance of the new polypeptide in each membrane fraction prepared from TM-treated tissue (Fig. 2, B, D, and F; arrow 7). Polypeptide No. 7, synthesized by intact soybean seedlings treated with TM for 15 h was unable to bind con A, suggesting that this polypeptide lacked terminal α-D-glucosyl and -mannosyl residues and internal 2-*O*-linked α-D-mannopyranosyl and -glucopyranosyl residues (Fig. 3). Furthermore, polypeptide No. 7 migrated on two-dimensional gels to a position just below polypeptide No. 6 (*cf.* Figs. 1 and 2, panels B, D, F; and Fig. 3B). Polypeptide No. 6 contained carbohydrate residues capable of binding con A (Fig. 3, C and D). These results suggest that polypeptide 7 was a nonglycosylated form of glycopolypeptide 6.

Chemical deglycosylation of proteins using TFMSA is a technique which avoids the problems of substrate specificity and steric hindrance inherent with an enzymic deglycosylation approach (6, 25). Although TFMSA treatment of glycopolypeptide No. 6 yielded a polypeptide of greater mobility in SDS gels, the treatment produced two different pI forms of the deglycosylated protein (Fig. 4). In contrast, *N*-glycanase yielded only a single polypeptide of the same pI as the untreated protein but of greater mobility in the SDS gel (Fig. 4). The additional polypeptide produced by the TFMSA procedure would not be detected on one-dimensional PAGE as was employed by Edge *et al.* (6) and Paaren *et al.* (25). The decrease in M_r for the deglycosylated membrane glycopolypeptide No. 6 was about 5000 (Fig. 4); similar to the decrease apparent between glycopolypeptide No. 6 on the amido black-stained electrophoretograms and polypeptide No. 7 on the autoradiograms (*cf.* Figs. 1 and 2; panels B, D, F) and clearly apparent when intact tissue was treated for 15 h (Fig. 3). The decrease in M_r represents about 10% of the mass and is in agreement with literature values of the percentage carbohydrate content of the relatively few glycoproteins characterized (29).

Mammalian cell surface proteins were previously shown to be transported to the cell surface in a nonglycosylated form (4). In yeast cells, the lack of saccharide moieties did not prevent the delivery of glycoproteins to their proper destination and it was postulated that glycosylation may influence rates of processing, turnover, and stability (19). Yet, the integral membrane glycoprotein of vesicular stomatitis virus remained in the GA in a nonglycosylated state but was transported to the cell surface if at least one of its two N-linked oligosaccharides was present (22). In a recent review, Pfeffer and Rothman (26) proposed that PM proteins must be (a) intercalated into the membrane of the ER and (b) freely mobile in the lipid bilayer to be transported through the GA to the PM. Resident ER luminal proteins appear to have a retention signal at their C-terminus comprised of the amino acid sequence lys-asp-glu-leu (23). Proteins destined for other cellular locations require a sorting signal putatively composed of noncontiguous regions of the linear polypeptide chain which form a signal patch upon folding of the polypeptide (26). The appearance of a 'nonglycosylated glycopolypeptide' (No. 7) in the GA and PM isolated from TM-treated root tissue suggests that the carbohydrate moieties of integral membrane proteins in plant cells may not function as sorting signals for transport to the PM. The present study does not provide the information necessary to determine if the polypeptide assumed its proper orientation in the lipid bilayer or retained biological functionality in the nonglycosylated form.

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