

Inhibition of Cell Wall-Associated Enzymes *in Vitro* and *in Vivo* with Sugar Analogs

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ABSTRACT

Sugar analogs were used to study the inhibition of cell wall-associated glycosidases *in vitro* and *in vivo*. For *in vitro* characterization, cell walls were highly purified from corn (*Zea mays* L.) root cortical cells and methods were developed to assay enzyme activity *in situ*. Inhibitor dependence curves, mode of inhibition, and specificity were determined for three sugar analogs. At low concentrations of castanospermine (CAS), 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol, and swainsonine, these inhibitors showed competitive inhibition kinetics with β -glucosidase, β -GlcNAcase, and α -mannosidase, respectively. Swainsonine specifically inhibited α -mannosidase activity, and 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol specifically inhibited β -N-acetylhexosaminidase activity. However, CAS inhibited a broad spectrum of cell wall-associated enzymes. When the sugar analogs were applied to 2 day old corn seedlings, only CAS caused considerable changes in root growth and development. To ensure that the concentration of inhibitors used *in vitro* also inhibited enzyme activity *in vivo*, an *in vivo* method for measuring cell wall-associated activity was devised.

Glycosyl derivatives, glycals, and glyconolactones have been useful for studying the active site and mechanism of catalysis of glycosidases and for investigating the physiological roles of glycosidases (9). Recently, the interest in sugar analogs has been rekindled because of their potential use in pest management (4) and their anti-acquired immune deficiency syndrome activity (19). Two naturally occurring compounds (indolizidine alkaloids) have been shown to inhibit soluble and membrane-bound glycosidases from plant (7, 16–18) and animal cells (16, 18). CAS¹, an inhibitor of α - and β -glucosidases, and SWA, an inhibitor of α -mannosidase, have been used to study glycoprotein processing (3). A third inhibitor, NAG, was recently synthesized (5) and has not been tested on plant β -N-acetylhexosaminidase activity.

These inhibitors have potential use in studies involving cell wall-associated glycosidases. Three roles for these glycosidases have been postulated. In storage tissue such as seeds, the enzymes may function in the mobilization of cell wall polysaccharides as carbohydrate reserves during seedling growth (1). Cell wall glycosidases may also be involved in cell wall loosening during elongation (6), or they may be a part of the

host defense system to prevent pathogen invasion (15). If it can be demonstrated that specific cell wall enzymes can be inhibited *in vitro* and *in vivo*, it will then be possible for future studies to elucidate their physiological roles.

MATERIALS AND METHODS

Plant Material

Corn (*Zea mays* L.) seeds (WF9 \times MO17) were surface-sterilized by stirring in diluted Clorox for 15 min at room temperature. The commercial bleach Clorox was diluted 1:10 just before use. Seeds were then washed in cold tap water for 30 min before planting in glass trays as previously described (10). Glass trays and filter paper were autoclaved before using. Under these conditions, no microbial growth was observed in the trays during seed germination. After 3 d, the root tips were excised, and the cortex was physically separated from the stele (10) to eliminate the vascular tissue. Preliminary experiments were performed with *Z. mays* FRB-73, but removal of the cortex from the stele was very difficult in this variety (11).

Cell Wall Isolation Procedures

The isolated cortices were frozen in liquid nitrogen, pulverized with a mortar and pestle, suspended in 300 mL of homogenization medium (0.3 M sucrose + 5 mM 2-mercaptoethanol + 2 mM Na₂S₂O₅ + 5 mM EDTA in 0.1 M HEPES buffer at pH 7.8), and placed in a Parr Nitrogen Bomb. This homogenization medium minimized the binding of cytoplasmic protein to cell walls during cell disruption (12). Contents of the bomb were allowed to equilibrate at 1500 p.s.i. for 10 min at 4°C (13). During the extrusion process, the pressure of the bomb was not allowed to drop below 1000 p.s.i. Crude cell walls were trapped on four layers of cheesecloth (40 \times 38 threads/inch²), washed twice with cold distilled water, suspended in fresh homogenization medium, and sonicated for 5 min at 4°C with a sonicator (Cell Disruptor Model-225R, Heat Systems-Ultrasonics, Inc.). Sonicator settings were at output control 7 with a 50% pulsed cycle. After sonication, the cell walls were washed six times with cold distilled water, and between washings the walls were trapped on the cheesecloth sieve. Aliquots of cell walls were prepared for electron microscope examination as previously described (13).

¹ Abbreviations: CAS, castanospermine; SWA, swainsonine; NAG, 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol; PNP, *p*-nitrophenol.

Assays

Glycosidase activity was determined as previously described (13) except the pH was varied as shown in the text. Aliquots of purified cell wall were dried at 110°C for 15 h to determine the dry weight of walls used in the enzyme assays. *In vivo* enzyme assays were performed by placing intact roots into 30 mL of various PNP-sugar substrates at room temperature or 38°C. Aliquots were removed at specified time intervals to determine the amount of product released. *In vivo* growth studies were performed by placing 2 d old seedlings in either water or various inhibitors. Initial observations, such as root length, were recorded and then made again after 24 h and 48 h. The inhibitors were either purchased from Sigma (CAS), donated by Dr. Russell Molyneux (SWA), or synthesized in Dr. Fleet's laboratory.

RESULTS AND DISCUSSION

Purity of Isolated Cortical Cell Walls

Light microscopy indicated that no intact cells were present after extrusion from the N₂ bomb followed by sonication. At the ultrastructural level, no membrane fragments, vesicles, or cytoplasmic fragments were observed in the purified cell wall fraction (data not shown). The purity was confirmed by lack of biochemical markers for the cytoplasm (malate dehydrogenase activity and triose phosphate isomerase activity) and membranes (Cyt *c* oxidase and NADH Cyt *c* reductase activity). Phospholipid analysis showed very little phosphatidylcholine associated with the purified cell walls (0.08% of the total original cortical homogenate).

In Situ Assays with Enzymes Bound to Walls

To determine the *in situ* properties of cell wall enzymes, a time course and cell wall dependence curve were first developed. The enzyme assays used in this study were linear up to 60 min with 10 to 30 min as the usual incubation time. Enzyme assays were also linear with respect to cell wall concentration between 0.1 to 0.7 mg dry weight when assayed for 10 min (Fig. 1A). To standardize *in situ* assays, the packed cell wall volume (determined by centrifugation at 1000g for 10 min in 5 mL graduated conical tubes) was compared to the dry weight of cell walls. The linear relationship between packed cell walls and oven dried weight (Fig. 1B) provided a simple method for estimating the dry weight for a cell wall suspension. Once the standard curve was constructed, any cell wall sample could be rapidly adjusted to an approximate dry weight by the addition or removal of H₂O. This provided a rapid, nondestructive method for adjusting the cell wall concentration for a particular enzyme assay. It should be noted that the slope of the standard curve will vary with the type of cell disruption step employed (size of fragments) and the type of plant tissue used (geometry of cells). For example, the standard curve for carrot cell walls was different from potato cell walls, and both of these (data not shown) were different from corn root cortical cell walls (Fig. 1B).

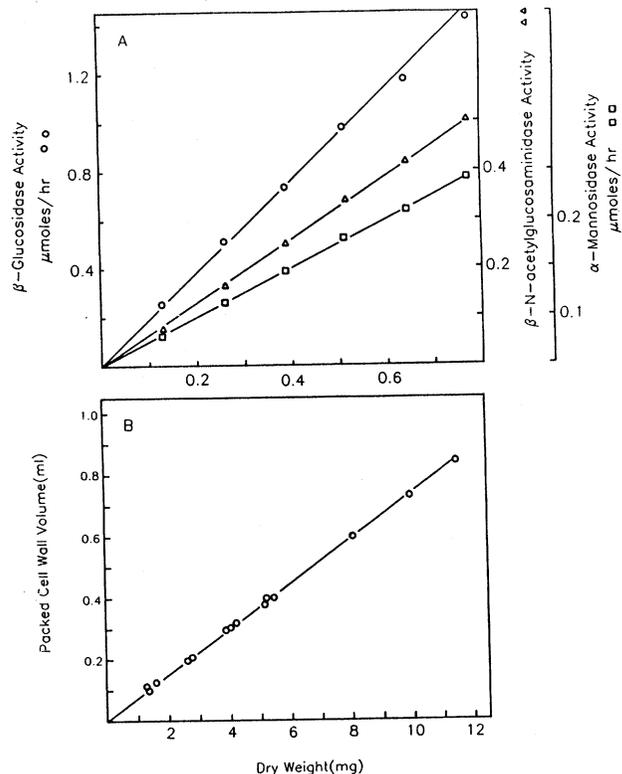


Figure 1. (A) Corn root cell wall dependence curves for *in situ* enzyme assays. (B) Cell wall standard curve that correlates packed cell wall volume (1000g for 10 min) with actual dry weight.

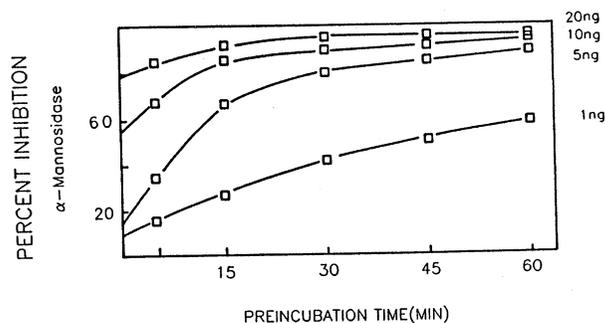


Figure 2. Preincubation effect of SWA on corn root cortical cell wall-associated α -mannosidase activity. Purified cell walls were preincubated in the presence of the inhibitor without substrate at room temperature. After the preincubation period, the enzyme was assayed from 20 min with PNP- α -mannose as substrate. Inhibitor concentration was ng/mL.

Inhibition of Cell Wall-Associated Enzymes *In Vitro*

For preincubation experiments, cell walls were incubated at room temperature in the presence of inhibitor and in the absence of substrate. At low concentrations of SWA, the degree of inhibition of the cell wall-associated α -mannosidase became greater as the preincubation time was increased (Fig. 2). As the concentration of SWA was increased, the preincubation time effect was considerably reduced (Fig. 2). A similar preincubation result was reported earlier for soluble α -man-

nosidase activity isolated from jack bean (7, 18), however; the preincubation period was much shorter (15 min) and the preincubation experiments were performed at a higher concentration (5–10 μM). In contrast, preincubation of cell walls with CAS or NAG showed no increase in inhibition with time for β -glucosidase and β -GlcNAcase activity, respectively (data not shown).

Inhibitor concentration curves for a broad range were then performed for all three sugar analogs. CAS and NAG both showed similar inhibited curves for their respective β -glucosidase (data not shown) and β -GlcNAcase activity (Fig. 3). At 25 $\mu\text{g}/\text{mL}$ of inhibitor, both enzymes were inhibited by over 90%. For SWA inhibition, a preincubation period was not used because the time-lag effect diminished with increasing concentration of SWA (Fig. 2). A 1000-fold less inhibitor (25 ng/mL) was necessary to achieve approximately 80% inhibition when compared to CAS and NAG (Fig. 3). In general, the cell wall-associated α -mannosidase was considerably more sensitive to SWA than was the soluble jack bean enzyme (18).

Inhibition Kinetics of Cell Wall-Associated Enzymes *in Situ*

If a 30 min preincubation of cell walls with SWA was used before enzyme assay, the kinetic plots were curvilinear (data not shown) as was the reported case for jack bean α -mannosidase (7). After a short preincubation, the jack bean α -mannosidase had curvilinear Lineweaver-Burk plots that could be extrapolated back to the $1/v$ axis at high substrate concentration where they intersected with the control. The authors (7) suggested SWA was a competitive type inhibitor. When kinetics of SWA inhibition were obtained for cell wall-bound α -mannosidase without a preincubation (Fig. 4), the Lineweaver-Burk analysis clearly showed SWA was a competitive inhibitor. The difference in kinetic results observed with and without preincubation was puzzling and could indicate that the cell wall has two different α -mannosidases that react

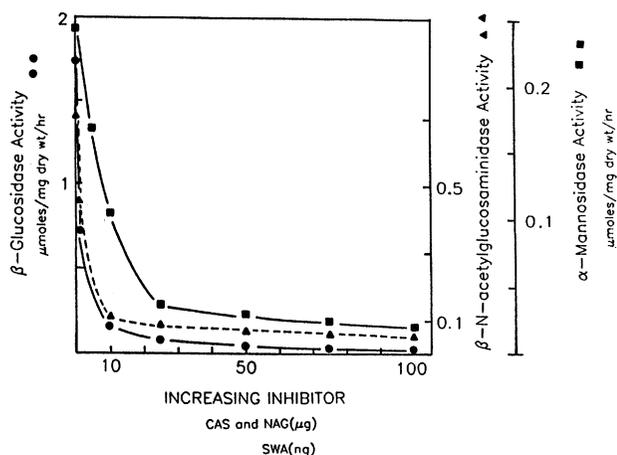


Figure 3. Inhibition dose dependence curves of various sugar analog on their respective corn root cortical cell wall-associated enzyme activity. NAG ($\mu\text{g}/\text{ml}$) was used to inhibit β -N-acetylglucosaminidase; CAS ($\mu\text{g}/\text{mL}$) was used to inhibit β -glucosidase; SWA (ng/mL) was used to inhibit α -mannosidase.

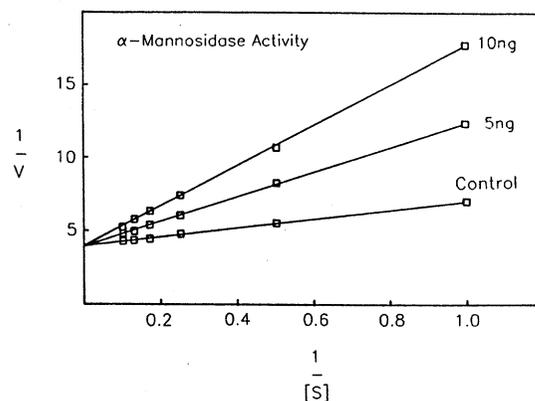


Figure 4. Lineweaver-Burk analysis of corn root cortical cell wall-associated α -mannosidase activity assayed *in situ*. Swainsonine was used as the inhibitor (ng/mL). Lines were fit by linear regression analysis with Sigma plot software.

differently to SWA, or there was only one α -mannosidase with two different modes of inhibition as was suggested earlier for liver Golgi mannosidase II and lysosomal α -D-mannosidase (18).

The inhibition of cell wall-associated β -glucosidase by CAS was clearly competitive in nature (data not shown) and this confirmed the earlier report with soluble almond emulsin β -glucosidase (17). The K_m and K_i values for the cell wall enzyme were considerably smaller (Table I) than those determined for the soluble enzyme. β -GlcNAcase was also competitively inhibited by NAG and at higher inhibitor levels the kinetic plots began to curve (Fig. 5). Curvilinear plots were also seen for CAS inhibition of the β -glucosidase (data not shown) under the conditions of higher inhibitor and low substrate concentration. Similar results have been reported previously for sugar analog inhibition of glycosidase activity (7, 8, 18; *cf.* 16 and 17). The nonlinearity of inhibition may be due to the fact that the K_i for glycosidases were usually much smaller than the K_m (Table I). Alternatively, the inhibition at higher inhibitor concentration may be more complicated (2). The non-competitive or mixed-type competitive kinetic plots may indicate that sugar analogs have a binding site other than the active site.

Specificity of the Various Inhibitors

SWA was very specific for the cell wall-associated α -mannosidase, and this result (Table II) confirmed the previous reports on cytoplasmic α -mannosidases. NAG was specific for β -N-acetylhexosaminidases with 92% inhibition for β -GlcNAcase and 80% inhibition of β -GalNAcase (Table II).

Unlike previous reports (16, 17), we found that CAS inhibited many different glycosidases with the greatest effect on β -glucosidase, α -arabinosidase, β -cellobiase, and β -xylosidase. The β -cellobiase activity may actually be the β -glucosidase hydrolyzing one glucose residue at a time. The β -galactosidase and α -glucosidase were inhibited approximately 50%, while the β -mannosidase was inhibited approximately 40%. The inhibition of three soluble enzymes, β -glucosidase, as well as α -glucosidase and β -xylosidase, by CAS has been reported

Table I. Biochemical Properties of Corn Root Cell Wall-Associated Enzymes

Cell walls were isolated and purified from cortical cells and pH optima and kinetic properties were determined with enzymes bound to the wall.

Enzyme Activity	pH Optimum	V_{max} $\mu\text{mol}/$ $\text{mg dry wt} \cdot \text{h}$	K_m mM	Inhibitor	K_i μM
β -Glucosidase	5.0–5.5	2.49	0.71	CAS	1.220
β -N-Acetylglucosaminidase	4.5–5.0	1.02	0.59	NAG	0.770
α -Mannosidase	4.5	0.76	0.25	SWA	0.016

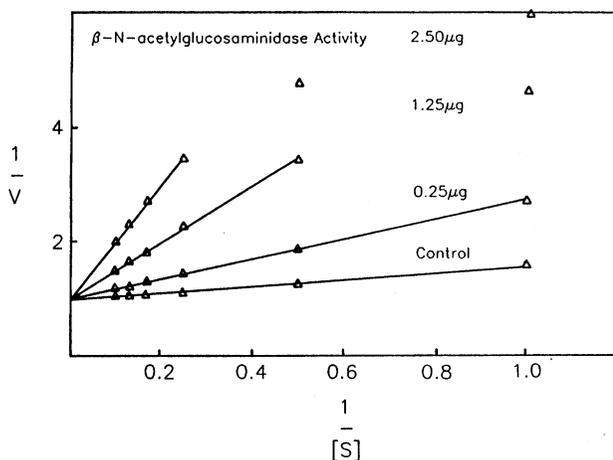


Figure 5. Lineweaver-Burk analysis of corn root cortical wall-associated β -N-acetylglucosaminidase activity assayed *in situ*. NAG was used as the inhibitor ($\mu\text{g}/\text{mL}$). Lines were fit by linear regression analysis with Sigma plot software.

earlier (16, 17). The broad specificity of the inhibitor we observed may not be restricted to cell wall-associated enzymes, but this has not yet been substantiated for soluble enzymes. CAS may be acting as a broad spectrum inhibitor because this compound has four hydroxyl groups and may be able to enter many different active sites.

In Vivo Measurements

Based on the results obtained from *in vitro* inhibition studies, 25 $\mu\text{g}/\text{mL}$ CAS, 25 $\mu\text{g}/\text{mL}$ NAG, and 25 ng/mL SWA were applied to intact corn roots after 2 d growth. Seedlings of similar size were selected, the length of the primary root was determined, and batches of intact roots were placed in solutions of various inhibitors or water as the control. Root length and other observations were made after 24 h and 48 h and the results were summarized in Table III. Only the roots grown in CAS showed visual differences compared to the control. After 48 h, the net growth in root length was inhibited by 50%, the zone of elongation was deformed (curled), and secondary roots did not form. Secondary root formation initiates from the pericycle and relies on the secretion of extracellular hydrolases to aid penetration through the cortical region. Apparently, CAS inhibited the extracellular glycosidases necessary for secondary root growth.

The distinct inhibition of growth caused by CAS could be due to the broad specificity of the inhibitor on cell wall-associated enzymes. Although it is assumed that sugar analogues only inhibit exoglycosidase activity (14), both exoglycosidase and endoglycosidase activity apparently are necessary for cell wall elongation of corn tissue (6). Alternatively, the large changes in growth caused by CAS could be due to the inhibition of cytoplasmic enzymes. CAS has been reported to inhibit glycoprotein processing in plant tissue (3), and at this

Table II. Specificity of Sugar Analog Inhibitors on Corn Root Cortical Cell Wall-Associated Glycosidase Activity

The data in the presence of inhibitor are expressed as a percentage of the activity in the absence of inhibitor (control). Concentrations of inhibitors were 50 $\mu\text{g}/\text{mL}$ CAS, 50 $\mu\text{g}/\text{mL}$ NAG, and 100 ng/mL SWA. All substrates were PNP-derivatives. Hydrolysis of PNP- β -galacturonide and PNP- β -glucuronide was very low even after 1 h incubation (data not shown).

Enzyme	Control $\mu\text{mol}/\text{mg dry wt} \cdot \text{h}$	Plus Inhibitor		
		NAG	CAS	SWA
α -Arabinosidase	0.119 (± 0.008)	102	3.2	105
β -Cellobiase	0.154 (± 0.003)	100	3.3	106
β -Galactosidase	0.116 (± 0.003)	100	52.1	103
α -Glucosidase	0.023 (± 0.001)	100	48.0	96
β -Glucosidase	2.127 (± 0.010)	100	4.6	105
β -N-Acetylgalactosaminidase	0.215 (± 0.009)	20.6	100	100
α -N-Acetylglucosaminidase	0.026 (± 0.001)	100	100	100
β -N-Acetylglucosaminidase	0.656 (± 0.007)	8.0	100	100
α -Mannosidase	0.268 (± 0.002)	100	100	6.4
β -Mannosidase	0.033 (± 0.002)	103	59.5	100
β -Xylosidase	0.070 (± 0.006)	100	21.4	100

Table III. Effects of Various Sugar Analogs on Growth of Corn Seedlings

Seedlings were grown for 2 d and selected for uniformity in length (40 seedlings out of 10 trays were selected). Root length was measured, and intact roots were placed in either water or inhibitor solution. Data shown are averages of 10 roots per treatment \pm standard deviation from the mean. The experiment was repeated twice with virtually identical results as those shown.

Treatment	Net Growth after		Secondary Root Formation	Zone of Elongation
	24 h	48 h		
	<i>mm</i>			
Control (H ₂ O)	33.2 \pm 2.5	62.8 \pm 4.0	+	Unaffected
+ CAS (25 μ g/ml)	24.8 \pm 3.1	30.4 \pm 3.6	-	Curled like a corkscrew
+ NAG (25 μ g/ml)	32.4 \pm 3.2	61.0 \pm 4.2	+	Unaffected
+ SWA (25 ng/ml)	35.1 \pm 3.4	68.8 \pm 4.5	+	Unaffected

time, it was not determined if cell wall enzyme inhibition, cytoplasmic enzyme inhibition, or a combination of both was necessary to inhibit growth in corn roots.

SWA and NAG had no observable effects after 48 h (Table III) and the possible changes in growth after a longer time period were not determined. Although α -mannosidase and β -GlcNAcase could be inhibited *in vitro*, it was conceivable that SWA and NAG were not inhibiting cell wall-associated activity *in vivo*. To resolve this question, an *in vivo* assay for cell wall-associated enzymes was devised.

Intact roots were submerged in substrate, and aliquots were removed at specific times to quantitate product released by enzymatic hydrolysis. The assumption was that two major compartments exist: the cell wall and the cytoplasm which were separated by a membrane. During a time course, the initial linear rate observed was due to cell wall-associated (first compartment) enzyme activity and the increase in rate with time (increase in the slope) was assumed to be from the additional product generated by cytoplasmic enzyme activity (second compartment). If the biphasic response was actually caused by two physically separated compartments, then intact roots pretreated with Triton X-100 should generate a single linear phase.

The *in vivo* assay for α -mannosidase (Fig. 6) was performed at 25°C and 38°C because *in vitro* activity was determined at 38°C. At 25°C, the phase change after 20 min was eliminated by pretreating intact roots with 0.5% Triton X-100 for 30 min. The generation of leaky membranes eliminated the compartmental effect, and this was substantiated by the fact that the cytoplasmic enzymes did not come out but the substrate readily entered the cells. Neither cytoplasmic enzymes nor the cell wall enzymes were activated by detergent *in vitro*. It should be noted that whenever Triton X-100 was used in *in vivo* enzyme assays, the reactions were terminated with 1 M NaOH instead of 1 M Na₂CO₃ because Na₂CO₃ plus detergent formed an insoluble precipitate that interfered with colorimetric analysis.

The β -GlcNAcase activity *in vivo* was also determined at 25°C and 38°C (Fig. 7). Both the β -GlcNAcase and α -mannosidase activities were higher at 38°C compared to 25°C (Figs. 6 and 7). The phase change was not as pronounced at the higher temperature, and the cell wall-associated enzymes of intact roots could be assayed for less than 10 min at 38°C to guarantee only measurement of cell wall activity (Figs. 6 and 7). The phase response was eliminated with 0.5% Triton

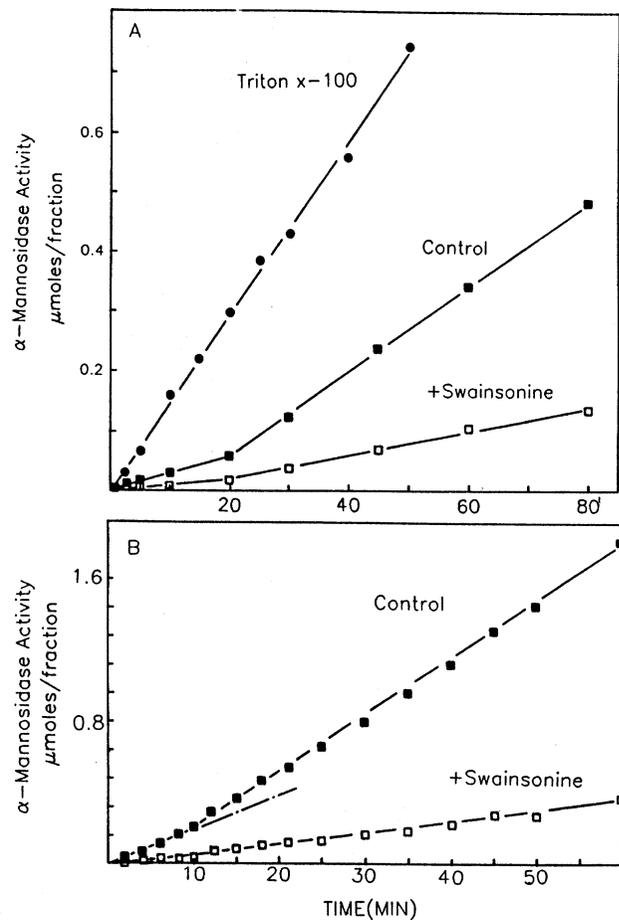


Figure 6. *In vivo* measurement of cell-associated α -mannosidase activity in intact corn roots. Assays were performed at 25°C (A) and 38°C (B). Forty roots were placed in 30 mL of substrate, and 0.3 mL aliquots were removed at specified times. Inhibitor concentration was 25 ng/mL (SWA). Assays were performed with (□) or without inhibitor (■). The biphasic response with increasing incubation time was due to the presence of two major compartments containing the α -mannosidase activity. The cell wall (first compartment) and the cytoplasm (second compartment) were separated by a membrane which could be made leaky by pretreating intact corn roots with 0.5% Triton X-100 for 30 min at room temperature (●)

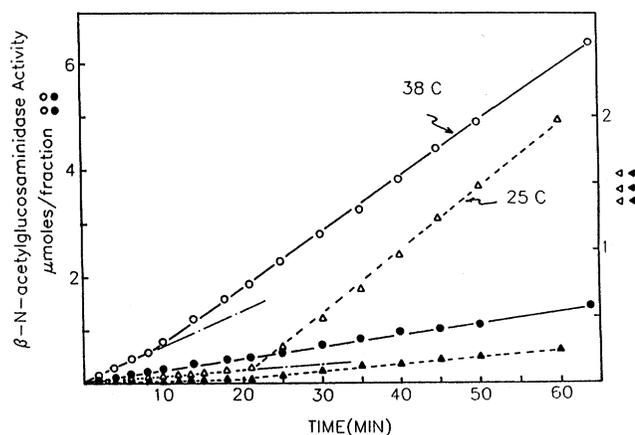


Figure 7. *In vivo* measurement of cell wall-associated β -N-acetylglucosaminidase activity in intact corn roots. Forty intact roots were placed in 30 mL of substrate and 0.3 mL aliquots were removed at the specified times. Assays were performed at 25°C plus (\blacktriangle) and minus (\triangle) 25 μ g/mL NAG or at 38°C plus (\bullet) and minus (\circ) NAG. For further details, see Figure 6.

X-100 at both temperatures. The cell wall enzymes were clearly inhibited *in vivo* at both temperatures (Figs. 6 and 7).

Both NAG and SWA clearly inhibited their respective cell wall-associated β -GlcNAcase and α -mannosidase activities (Figs. 6 and 7) *in vivo* and yet showed no observable changes in growth after 48 h (Table III). Because growing corn roots are not considered as a storage tissue, it is unlikely that root cell wall-associated glycosidases are involved in mobilization of carbohydrate reserves. It is also unlikely that the β -GlcNAcase and α -mannosidase are involved in cell wall elongation or cell wall turnover. Therefore, future studies on the physiological role of these two enzymes should probably be focused on the host-defense system. These enzymes may either hydrolyze fungal wall polysaccharides or hydrolyze the sugar residues of fungal secreted glycoproteins.

LITERATURE CITED

- Crawshaw LA, Reid JSG (1984) Changes in cell-wall polysaccharides in relation to seedling development and mobilization of reserves in the cotyledons of *Lupinus angustifolius* cv. Unicorp. *Planta* **160**: 449-454
- Dorling PR, Huxtable CR, Colegate SM (1980) Inhibition of lysosomal α -mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *Biochem J* **191**: 649-651
- Elbein AD (1988) Glycoprotein processing and glycoprotein processing inhibitors. *Plant Physiol* **87**: 291-295
- Evans SV, Fellows LE, Shing TKM, Fleet GWJ (1985) Glycosidase inhibition by plant alkaloids which are structural analogues of monosaccharides. *Phytochemistry* **24**: 1953-1955
- Fleet GWJ, Fellows LE, Smith PW (1987) Synthesis of deoxymannojirimycin, fagomine, deoxynojirimycin, 2-acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol, 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol, 2S,3R,4R,5R-trihydroxypipercolic acid and 2S,3R,4R,5S-trihydroxypipercolic acid from methyl 3-O-benzyl-2,6-dideoxy-2,6-imino- α -D-mannofuranoside. *Tetrahedron* **43**: 979-990
- Inouhe M, Nevins D (1988) Evidence for the involvement of cell wall autolysis in auxin-controlled glucan metabolism and cell extension (abstract No. 571). *Plant Physiol* **86**: S-95
- Kang MS, Elbein AD (1983) Mechanism of inhibition of jack bean α -mannosidase by swainsonine. *Plant Physiol* **71**: 551-554
- Kuroki G, Poulton JE (1986) Comparison of kinetic and molecular properties of two forms of amygdalin hydrolase from black cherry (*Prunus serotina*) seeds. *Arch Biochem Biophys* **247**: 433-439
- Lalegerie P, Legler G, Yon JM (1982) The use of inhibitors in the study of glycosidases. *Biochimie* **64**: 977-1000
- Leonard RT, Nagahashi G, Thomson WW (1975) Effect of lanthanum on ion absorption in corn roots. *Plant Physiol* **55**: 542-545
- Nagahashi G, Barnett PM, Tu S-I, Brouillette J (1986) Purification of primary cell walls from corn roots: inhibition of cell wall-associated enzymes with idolizidine alkaloids. In JC Shannon, DP Knievel, CD Boyer, eds, *Regulation of Carbon and Nitrogen Reduction and Utilization in Maize*. American Society of Plant Physiologists, Rockville, MD, pp 289-293
- Nagahashi G, Garzarella L (1988) An extrinsic marker for higher plant cell walls. *Protoplasma* **143**: 165-169
- Nagahashi G, Seibles TS (1986) Purification of plant cell walls: isoelectric focusing the CaCl_2 extracted enzymes. *Protoplasma* **134**: 102-110
- Nevins DJ (1975) The effect of nojirimycin on plant growth and its implications concerning the role for exo- β -glucanases in auxin-induced cell expansion. *Plant Cell Physiol* **16**: 347-356
- Pegg GF (1977) Glucanohydrolases of higher plants: A possible defence mechanism against parasitic fungi. In B Solheim, J Raa, eds, *Cell Wall Biochemistry Related to Specificity in Host-Pathogen Interactions*. Scandinavian University Books, Stockholm, pp 305-345
- Saul R, Chambers JP, Molyneux RJ, Elbein AD (1983) Castanospermine, a tetrahydroxylated alkaloid that inhibits β -glucosidase and β -glucocerebrosidase. *Arch Biochem Biophys* **221**: 593-597
- Saul R, Molyneux RJ, Elbein AD (1984) Studies on the mechanism of castanospermine inhibition of α - and β -glucosidase. *Arch Biochem Biophys* **230**: 668-675
- Tulsiani DRP, Broquist HP, Touster O (1985) Marked differences in the swainsonine inhibition of rat liver lysosomal α -D-mannosidase, rat liver Golgi mannosidase II and jack bean α -D-mannosidase. *Arch Biochem Biophys* **236**: 427-434
- Walker BD, Kowalski M, Goh WC, Kozarsky K, Krieger M, Rosen C, Rohrschneider L, Haseltine WA, Sodroski J (1987) Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. *Proc Natl Acad Sci USA* **84**: 8120-8124