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Inhibition of Auxin-Induced Cell Elongation of Maize Coleoptiles by Antibodies Specific for Cell Wall Glucanases[1]

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ABSTRACT

Polyclonal antibodies were raised in rabbits in response to the administration of purified exo- and endoglucanases extracted from cell walls of maize (*Zea mays* L. B37 × Mo17) coleoptiles. Since the antibodies formed specific conjugates when challenged with the glucanase antigens in immunoblot assays they were employed to evaluate the participation of glucanases in tissue growth. Indole-3-acetic acid induced cell elongation of abraded coleoptile segments was inhibited when the antibodies were supplied as a short term pretreatment (25–200 microgram/milliliter of serum protein). The extent of inhibition of IAA induced cell elongation was additive when endo- and exoglucanase antibodies were applied together. The results suggest that both enzymes have a role in mediating IAA-induced cell elongation. Pretreatment with exo- and endoglucanases antibodies also inhibited IAA induced degradation of noncellulosic β-D-glucans and the increased level of cellulosic polymers in maize coleoptiles. Antibodies also inhibited the expression of the autohydrolytic degradation of glucans in isolated cell walls. The extent of inhibition was dependent on the antibody concentration applied. The results support the contention that enzymatic processes mediated by exo- and endoglucanases are responsible for cell wall autolytic reactions and that these reactions are linked to the mechanism for expressing auxin induced cell elongation in maize coleoptiles.

Noncellulosic β-D-glucans of cereal coleoptile cell walls are metabolized during cell extension (16, 17, 22–24, 26). While the precise role of glucan metabolism is not clear the process is governed by auxin and the key metabolic steps may relate directly to cell wall changes required for cell elongation (18–20). Cell walls isolated from maize coleoptiles also exhibit autolysis resulting in the specific degradation of noncellulosic glucans. These autolytic reactions are mediated by exo- and endoglucanases (9). A recent study of the initial rates of cell wall glucan autolysis in maize coleoptiles demonstrates that an auxin pretreatment of segments stimulates the subsequent expression of cell wall autolysis (13).

To establish a relationship between auxin-mediated glucan metabolism and cell extension, molecular probes would serve to target specific events. In previous studies nojirimycin was applied for this purpose because of its capacity to inhibit specific enzymes (20). Nojirimycin also suppressed both auxin-induced glucan degradation *in vivo* and cell elongation. However, the development of antibodies offers an opportunity to conduct experiments that would complement the interpretation of results of experiments where inhibitors act to mimic substrates. Huber and Nevins (10) had first examined the physiological activity of antibodies generated in response to the administration of the LiCl extract of cell wall proteins to rabbits. These antibodies suppressed glucan autolysis and cell elongation of maize coleoptiles. An extension of that approach using more refined protein fractions reveals that only three maize cell wall proteins generate antibodies with the capability of inhibiting growth and for one of these a capacity for enzymatic activity has yet to be identified. Antibodies raised in response to specific wall glucanases offers an attractive approach for resolving their participation in key steps of auxin responses (8).

We evaluated the role of cell wall glucanases in glucan metabolism and in IAA-induced cell elongation of maize coleoptiles using antibodies raised against purified exo- and endoglucanases.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* L., hybrid B37 × Mo17) caryopses were allowed to imbibe for 24 h in running water at 27°C and sown on moist vermiculite in plastic trays. The seedlings were maintained under red light for 24 h and then placed in darkness for the subsequent 48 h at 26°C. The segments were cut when the coleoptiles had achieved a length of about 35 mm. Excised segments were pooled and held in distilled water for about 1 h.

Evaluation of Elongation

Suppression of elongation by antibodies was studied as reported previously (21). The outer surface of the coleoptile was abraded longitudinally with moist Carborundum (320 mesh). The abraded coleoptiles were rinsed with distilled water and 10 mm segments, with leaves removed, were cut from regions beginning 3 mm below the tip. The sections were placed in 10 mM K-citrate phosphate buffer (pH 6.5) containing 12.5 to 200 $\mu g \cdot mL^{-1}$ serum protein for a period of 1 h. After preincubation, the segments were rinsed with

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distilled water, a 5 mm subsection was excised and the 2.5 mm end pieces discarded. The 5 mm segments were treated with or without 30 μM IAA in 10 mM K-citrate phosphate (pH 6.5) buffer. The lengths of the segments were measured with the aid of a binocular microscope equipped with an ocular micrometer.

Purification of Cell Wall Glucanases

Cell wall glucanases were purified from maize coleoptile tissue according to methods of Huber and Nevins (11) as modified by Hatfield and Nevins (5). The population of proteins was extracted with 3 M LiCl from cell walls previously rinsed with 0.5 M NaCl and cold acetone (−20°C). The protein liberated by this procedure was dialyzed against Na-acetate buffer (20 mM, pH 5.4) containing 20 mM NaCl and concentrated by ultrafiltration (Amicon PM-10 membrane). The concentrated protein solution was applied to a SP-Sephadex column (0.9 × 15 cm) equilibrated with 20 mM Na-acetate buffer containing 20 mM NaCl. Then the column was eluted with the same buffer. Proteins including the major cell wall glucanases retained by the column were subsequently eluted with 500 mM NaCl in 20 mM Na-acetate (pH 5.4). After dialysis and concentration the active fractions were rechromatographed on SP-Sephadex (0.9 × 30 cm) employing a linear gradient of NaCl (100–500 mM) in 20 mM Na-acetate (pH 5.4) at a flow rate of 0.7 $mL \cdot min^{-1}$; the total volume used for elution was 500 mL. Separate exo- and endoglucanase fractions were collected and dialyzed against 20 mM K-citrate phosphate (pH 5.4) containing 0.2 M NaCl. The dialyzate was concentrated by ultrafiltration to reduce the volume to 2 mL and it was then subjected to Bio-Gel P-150 gel exclusion chromatography (1.8 × 140 cm). Elution was in the presence of the same buffer containing 0.2 M NaCl. The collected fractions comprised of exo- and endoglucanses were consolidated and rechromatographed on the P-150 column. Those fractions of the profile identified as individual glucanases were combined, dialyzed against the phosphate buffer solution, and stored at −20°C until used.

Preparation of Glucanase Antibodies

Antibodies were raised in female New Zealand rabbits by administration of isolated proteins as reported earlier (10, 21). Purified exo- and endoglucanases were separately emulsified with Freund's incomplete adjuvant (Difco Labs.) and subcutaneously injected on a weekly schedule with increasing dosages of protein (10–60 μg). Eight weeks after the first injection, blood was collected and sampling continued thereafter at 2 week intervals. The blood was maintained at 4°C for 12 h after which the serum was centrifuged at 10,000*g* to remove insoluble materials. The IgG in the supernatant was applied to a Protein A-Sepharose CL-4B column (Pharmacia). The IgG collected was dialyzed against Na-phosphate buffer at 4°C and 0.5 mL-aliquots were stored at −20°C. Immediately before use, the solution containing IgG was adjusted to the appropriate treatment buffer by Sephadex G-25 chromatography (NAP 5, Pharmacia).

SDS-PAGE and Western Blots of Cell Wall Proteins

Extracted cell wall proteins and purified glucanases (about 60 μg) were heated at 100°C for 1.5 min in the presence of 0.1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, and 10% glycerol (15). The solutions (1–10 μL) containing denatured proteins (1–10 μg) were applied to a SDS-PAGE slab gel (0.75 × 100 × 150 mm) and run for 8 to 10 h at 4°C at 10 amps. In general, gels were cut longitudinally through the center. One-half of the gel was fixed in 50% methanol and stained with nickel (nickel staining kit, Kodavue Electrophoresis Visualization Kit). The other half was subjected to immunoblot analysis (1). Proteins separated on the SDS-PAGE gel were electrically transferred to nitrocellulose paper by imposing 90 mV for 45 min. The nitrocellulose paper was then treated with appropriate antibodies and then with goat anti-rabbit IgG conjugated to peroxidase. Those materials transferred to nitrocellulose were identified by staining with a Bio-Rad kit. Biotinylated standard proteins (low mol wt Kit, Bio-Rad) were used as protein markers (3). In some experiments, antibodies raised against the total extracted cell wall proteins were used as first antibodies.

Determination of *in Vivo* and *in Vitro* Degradation of Cell Wall Glucans

Coleoptile segments were pretreated with antibodies for 1 h and then with IAA for 6 h. The glucan content of cell walls was determined as described previously (16). The cell walls were hydrolyzed with 2 M TFA for 1.5 h and the glucose content in the total sugars released was determined by GLC (16). An estimate of the cellulose content was based on analysis of the sugars (4) remaining after walls were subjected to 2 M TFA hydrolysis (16).

The rate of *in vitro* cell wall autolysis was determined as reported previously (13). Cell walls prepared immediately from 20 coleoptile segments that had been treated with and without auxin were packed into a small column (Bio-Rad) and washed with cold acetone (−20°C) and 50 mM NaCl (13). The walls were then incubated for 1 h with and without antibodies in 10 mM phosphate buffer (pH 5.4) and the autolytic capacity was determined from the release of sugars during subsequent 2 h intervals. The phenol sulfuric acid method (4) was used for sugar determinations.

RESULTS

Properties of Cell Wall Glucanases

Cell wall proteins extracted from maize coleoptiles retained substantial activity directed toward the degradation of *Avena* glucans (Fig. 1). Two enzymes were separated and purified from the LiCl extract of cell wall protein on a SP-Sephadex column (Fig. 1A) followed by gel permeation chromatography (Fig. 1, B and C). The action pattern of the enzymes was consistent with that of an exo- and an endoglucanase (5, 11). After the initial steps, the specific activities of these enzymes was not enhanced by further chromatographic separation employing either hydroxylapatite or DEAE-Sephadex (data not shown). After rechromatography of the glucanases on a Bio-Gel P-150 column single bands were resolved on SDS-

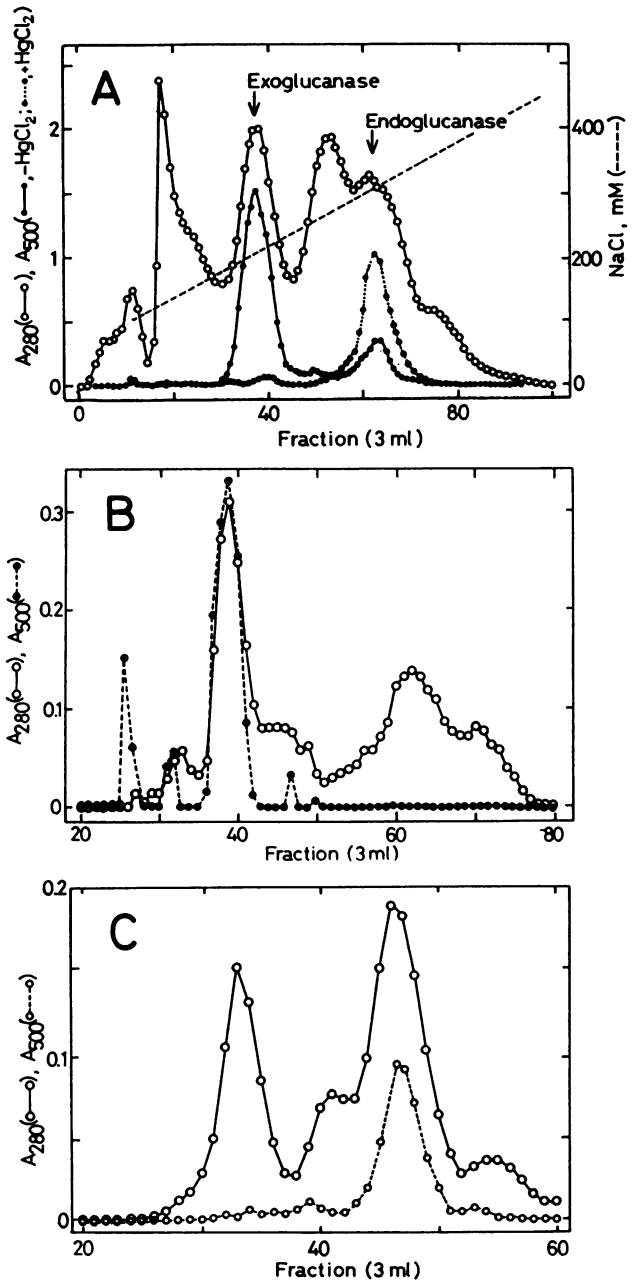

Figure 1. Isolation of maize coleoptile glucanases. Maize coleoptile cell wall proteins were extracted with 3 M LiCl. The proteins absorbed to SP-Sephadex were eluted employing a linear gradient of NaCl (100–500 mM) (A). Exo- and endoglucanase fractions were collected and separately chromatographed on a Bio-Gel P-150 column (B and C). Protein content was monitored by UV absorbance at 280 nm. Glucanase activities were assayed by measuring the increase in reducing groups in the presence of *Avena* β-D-glucan with (●– – –●) and without (●——●) 100 μM $HgCl_2$ (5, 11), Figure 1A. For Figure 1, B and C, the assays were conducted without Hg.

PAGE slab gels (Fig. 2). The molecular masses of the exo- and endoglucanases as estimated by SDS-PAGE were 74 and 33 kD, respectively. Increasing the endoglucanase protein loading onto the gel reveals a lighter band at about 31 kD.

Antibodies generated in rabbits in response to the injection of purified exo- and endoglucanases exhibited specific binding to the corresponding glucanases and the IgGs did not associate with other cell wall proteins (Fig. 3). However, when excess wall proteins were loaded on gels for immunoblot assays, limited binding was detected in the region adjacent to the endoglucanase band. These data suggest that exo- and endoglucanases are capable of inducing specific antibodies in rabbits.

Effect of Antibodies on IAA-Induced Cell Elongation

Preimmune serum from rabbits does not suppress the auxin-induced elongation responses of coleoptile segments and antibodies of only a few proteins extracted from walls have effects on the physiological responses under evaluation (8). However, the antibodies of both the exo- and the endoglucanase significantly suppress auxin induced elongation of segments (Fig. 4). The extent of inhibition was dependent upon the antibody concentration (between 0 and 200 $\mu g \cdot mL^{-1}$ of serum protein) even when exposure was in short term preincubations. When the exo- and endoantibodies were applied together, the inhibition of the IAA induced growth was additive. Over a range of concentrations the same quantity of serum protein was effective for the inhibition of growth regardless of whether applied separately or in a 1:1 ratio of the two (Fig. 5). This result suggests that both enzymes contribute to the response to auxin. Growth suppression was not observed when the IgG preparations were precipitated by their respective antigens, and the physiological effects were

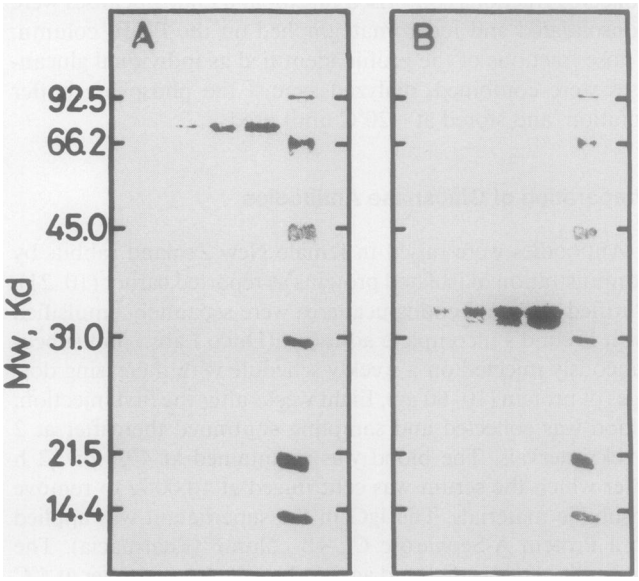

Figure 2. SDS-PAGE of purified cell wall glucanases. A, Exoglucanase; B, endoglucanase. In lanes 1, 2, 3, and 4 from the left to right 0.5, 1, 2, and 4 μg of protein were applied. Lane 5 at the far right of each gel represents the electrophoretic pattern of mol wt standards.

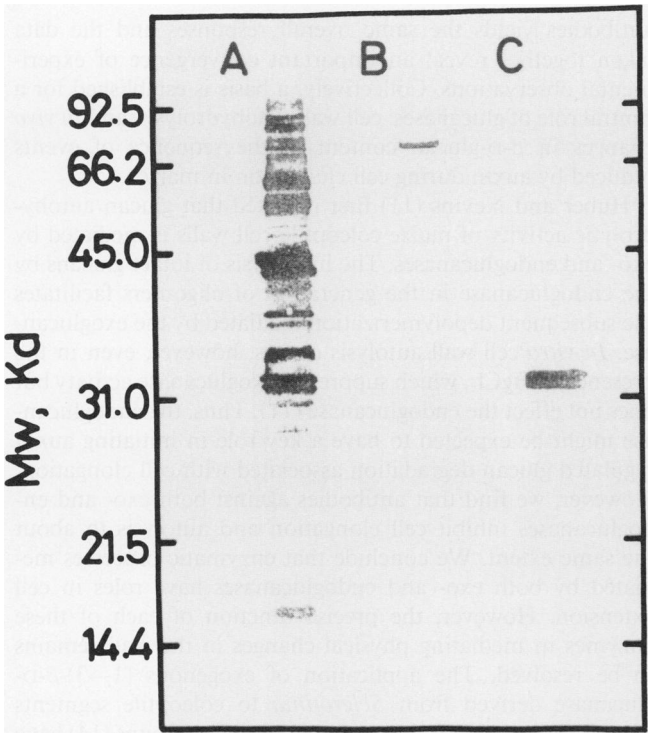

Figure 3. Immunoblot analysis of cell wall proteins with exo- and endoglucanase antibodies. Proteins extracted with LiCl were separated on SDS-PAGE in three lanes and transferred to nitrocellulose paper. The nitrocellulose paper was cut longitudinally into three sections. Each section was subjected to a different antibody preparation. Lane A was subjected to antibodies generated in response to the LiCl extracted proteins; lane B was subjected to antibodies generated in response to the purified exoglucanase; and lane C was subjected to antibodies generated in response to the purified endoglucanase.

not reversed by the presence of 50 mM sucrose or glucose in the incubation medium (data not shown).

Effect of Antibodies on Glucan Metabolism of Cell Walls

Antibodies influence the level of both cellulosic and noncellulosic glucans of maize cell walls (Fig. 6). Segments subjected to pretreatments with and without antibodies for 1 h were allowed to incubate in the presence and absence of IAA for 6 h. In segments not subjected to the exo- or endoglucanase antibodies, IAA caused a substantial decrease in noncellulosic glucan content and a somewhat elevated level of cellulosic glucan. These IAA effects were suppressed by pretreatments with the antibodies.

Cell walls freshly prepared from coleoptile tissues were pretreated with antibodies for 1 h and then allowed to autolyze for 2 h in a reaction buffer. The autolysis rate was inhibited by the presence of both exo- and endoglucanase antibodies (Fig. 7). Both the constitutive and auxin enhanced autolysis rates were affected. In a complementary experiment we were able to show that the inhibitory effect on autolysis could be imposed when antibodies were applied to coleoptile segments before homogenization. These experiments demonstrate that

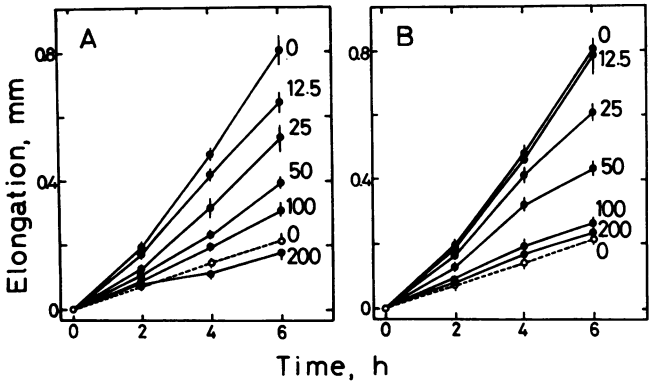

Figure 4. Inhibition of IAA-induced cell elongation in maize coleoptile segments by exo- and endoglucanase antibodies. For each treatment 20 abraded coleoptile segments (10 mm) were pretreated in the presence of 10 mM K-citrate phosphate buffer (pH 6.5) containing 12.5 to 200 $\mu g \cdot mL^{-1}$ serum protein antibodies for 1 h. The segments were rinsed in water, recut to 5 mm sections and treated with 30 μM IAA. A, Pretreated with exoglucanase antibodies for 1 h; B, pretreated with endoglucanase antibodies for 1 h. (O– – –O), Control, without IAA and in the absence of antibodies; (●——●), with IAA but in the absence of antibodies. The vertical lines indicate ± SE.

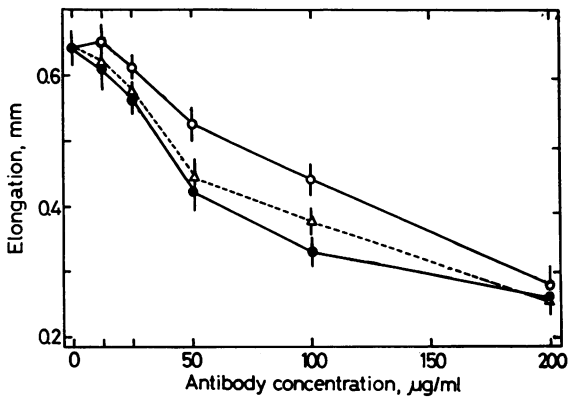

Figure 5. An assessment of the complementary effects of exo- and endoglucanase antibodies on cell elongation. Coleoptile segments were pretreated with exo- and endoglucanase antibodies individually or combined at a 1:1 ratio of serum protein for 1 h. The total amount of serum protein was constant for the three treatments at each concentration. The overall range of concentrations was selected based on the results as shown in Figure 4. Coleoptile segments were then allowed to incubate for 6 h in the presence of IAA. Otherwise, the treatment protocol was similar to that in Figure 4. Pretreatment with exoglucanase antibodies (●——●); pretreatment with endoglucanase antibodies (O——O); pretreatment with a 1:1 ratio of combined antibodies (△– – –△).

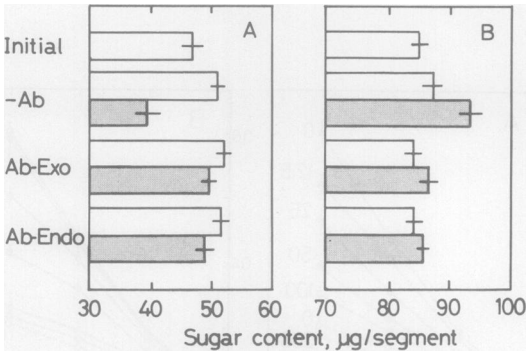

Figure 6. Effect of glucanase antibodies on the cell wall noncellulosic and cellulosic glucans. The coleoptile segments were pretreated with glucanase antibodies and then incubated in the presence or absence of IAA for 6 h. Cell walls were prepared from the segments. The wall noncellulosic glucans (A) were determined by GLC after 2 M TFA hydrolysis and the cellulosic glucans (B) were estimated by subsequent analysis of the wall residue by the phenolsulfuric acid method. The shaded and open bars in the histograms represent segments treated with and without IAA, respectively. The error bars represent ± SE.

antibodies can affect autolysis directly during the course of the treatment or as a pretreatment of intact segments.

DISCUSSION

Specific structural changes in cell wall constituents have been sought to account for adjustments in mechanical properties that accommodate auxin induced elongation. As a result of these studies we have gained an appreciation for the complexity of the wall structure and its integrated functions. However, the resolution of a structure/function relationship of this dynamic and highly ordered matrix in cell elongation has proved elusive. In cereal coleoptiles, the most studied system, auxin is responsible for initiation of the enhanced degradation of noncellulosic β-D-glucans (16), decreases in the mol wt of the polymer complex (12, 24), and the putative turnover of glucuronoarabinoxylans (2). For the evaluation of the function of these various changes in growth of Poaceae coleoptiles, an analysis employing specific molecular probes to inhibit or alter critical steps within the sequence of events is needed.

Polyclonal antibodies raised against cell wall proteins have been used for investigation of the role of selected cell wall components in extension growth (6, 8, 10). Antibodies, obtained from rabbits after injection with maize coleoptile wall protein fractions, strongly inhibit elongation of maize coleoptile segments and suppress wall β-D-glucan autolysis (10). Hoson and Nevins (7) have further demonstrated that antibodies raised in response to the administration of *Avena* (1→3),(1→4)-β-D-glucans to rabbits significantly inhibits IAA-induced cell elongation and wall autohydrolysis of maize coleoptiles. The current results demonstrate that antibodies prepared against exo- and/or endoglucanases purified from maize cell walls also strongly inhibit IAA induced elongation and wall β-D-glucan autolysis. Depressing enzyme activity by specific antibodies or protecting the substrate by antiglucan antibodies yields the same overall responses and the data taken together reveal an important convergence of experimental observations. Collectively, a basis is established for a central role of glucanases, cell wall autohydrolysis, and *in vivo* changes in β-D-glucan content in the sequence of events induced by auxin during cell elongation in maize.

Huber and Nevins (11) first reported that glucan autohydrolytic activity of maize coleoptile cell walls is mediated by exo- and endoglucanases. The hydrolysis of intact glucans by the endoglucanase in the generation of oligomers facilitates the subsequent depolymerization mediated by the exoglucanase. *In vitro* cell wall autolysis occurs, however, even in the presence of $HgCl_2$, which suppresses exoglucanase activity but does not effect the endoglucanase (11). Thus, the endoglucanase might be expected to have a key role in initiating auxin regulated glucan degradation associated with cell elongation. However, we find that antibodies against both exo- and endoglucanases inhibit cell elongation and autolysis to about the same extent. We conclude that enzymatic processes mediated by both exo- and endoglucanases have roles in cell extension. However, the precise function of each of these enzymes in mediating physical changes in the wall remains to be resolved. The application of exogenous (1→3)-β-D-glucanase derived from *Sclerotinia* to coleoptile segments stimulates growth (19, 25) and Labrador and Nevins (14) have reported that a limited but consistent enhancement of maize coleoptile elongation is mediated by application of the purified maize exoglucanase. The restricted enhancement of elongation in the presence of the maize exoglucanase may be indicative of the inherent limitation resulting from the application of a single enzyme, and the observations do not exclude an important role for the endoglucanase in cell extension. Moreover, one must remain cognizant that the addition of the exogenous enzymes would be expected to enhance hy-

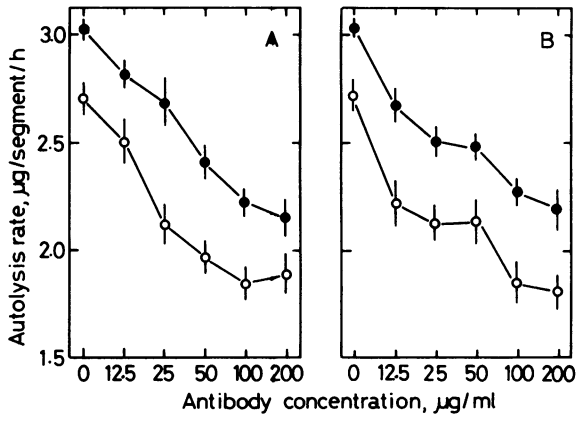

Figure 7. Effect of glucanase antibodies on cell wall autolysis. Freshly prepared cell walls from 20 coleoptile segments pretreated with and without IAA were treated with different concentrations of exoglucanase antibodies (A) or endoglucanase antibodies (B) for 1 h. The cell walls were then allowed to autolyze for 2 h at 37°C in columns as described in the text. The released glucose was determined by the phenol-sulfuric acid method. (●) represents sections pretreated with auxin for 2 h and (○) represents sections maintained in buffer for the same interval. The vertical lines represent ± SE.

drolysis in a background of constitutive enzymes. *In situ* regulation of enzyme activity by other mechanisms may be an important factor.

These studies demonstrate that antibodies raised against glucanases bind specifically to their corresponding antigens. The specificity is supported by the absence of inhibitory effects induced by treatment with preimmune serum and experiments that show that the inhibitory effects of the IgG fraction may be neutralized after selective precipitation by cross-reaction with appropriate antigens. Therefore, the inhibitory effects caused by these antibodies on growth and autolysis are a direct or indirect consequence of the suppression of the activity of target enzymes.

Because endoglucanase antibodies may react (based on immunoblot in the presence of excess protein) albeit weakly with the exoglucanase, the two enzymes may have epitopes in common. In contrast, however, we find no evidence that antibodies raised against the exoglucanase have the capacity to react with the endoglucanase even when challenged with excess protein. The exoglucanase may be a modified form of the endoglucanase with perhaps some common genomic origins. This hypothesis is to be evaluated by sequence analysis of the two enzymes and a more stringent antigenic comparison utilizing monoclonals.

In summary, the results point to a role for specific glucanases in cell wall metabolism associated with auxin mediated growth. Furthermore evidence for the interdependency of two enzymes, the cell wall exoglucanase and endoglucanase, suggests that a molecular mechanism responsible for altering the mechanical properties of the wall may be complex. Nevertheless, the application of antibodies offers new avenues for probing the regulation of cell wall β-D-glucan metabolism and to explore its role in cell elongation mediated by auxin.

LITERATURE CITED

1. **Burnette WN** (1981) "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radioactive protein A. Anal Biochem **112:** 195–203
2. **Darvill AG, Smith CJ, Hall MA** (1978) Cell wall structure and elongation growth in *Zea mays* coleoptile tissue. New Phytol **80:** 503–516
3. **Dellapenna D, Christofferson RE, Bennett AB** (1986) Biotinylated proteins as molecular weight standards on Western blots. Anal Biochem **152:** 329–332
4. **Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F** (1956) Colorimetric method for determination of sugars and related substances. Anal Chem **28:** 350–356
5. **Hatfield R, Nevins DJ** (1986) Purification and properties of an endoglucanase isolated from the cell walls of *Zea mays* seedlings. Carbohydr Res **148:** 265–278
6. **Hatfield R, Nevins DJ** (1988) Plant cell wall proteins: partial characterization of maize wall proteins with putative roles in auxin-induced growth. Plant Cell Physiol **29:** 713–720
7. **Hoson T, Nevins DJ** (1989) β-D-Glucan antibodies inhibit auxin-induced cell elongation and changes in the cell wall of *Zea* coleoptile segments. Plant Physiol **90:** 1353–1358
8. **Hoson T, Nevins DJ** (1989) Effect of anti-wall protein antibodies on auxin-induced elongation, cell wall loosening, and β-D-glucan degradation in maize coleoptile segments. Physiol Plant **77:** 208–215
9. **Huber DJ, Nevins DJ** (1980) β-D-glucan hydrolyase activity in *Zea* coleoptile cell walls. Plant Physiol **65:** 768–773
10. **Huber DJ, Nevins DJ** (1981) Wall-protein antibodies as inhibitors of growth and autolytic reactions of isolated cell wall. Physiol Plant **53:** 533–539
11. **Huber DJ, Nevins DJ** (1982) Exoglucanases from *Zea mays* L. seedlings: their role in β-D-glucan hydrolysis and their potential role in extension growth. Planta **155:** 467–472
12. **Inouhe M, Yamamoto R, Masuda Y** (1984) Auxin-induced changes in the molecular weight distribution of cell wall xyloglucans in *Avena* coleoptiles. Plant Cell Physiol **25:** 1314–1351
13. **Inouhe M, Nevins DJ** (1991) Auxin-enhanced glucan autohydrolysis in maize coleoptile cell walls. Plant Physiol **96:** 285–290
14. **Labrador E, Nevins DJ** (1989) An exo-β-D-glucanase derived from *Zea* coleoptile walls with a capacity to elicit cell elongation. Physiol Plant **77:** 479–486
15. **Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
16. **Loescher W, Nevins DJ** (1972) Auxin-induced changes in *Avena* coleoptile cell wall composition. Plant Physiol **50:** 556–563
17. **Luttenegger D, Nevins DJ** (1985) Transient nature of a (1→3),(1→4)-β-D-glucan in *Zea mays* coleoptile cell walls. Plant Physiol **77:** 175–178
18. **Masuda Y** (1978) Auxin-induced cell wall loosening (special issue). Bot Mag **1:** 103–123
19. **Masuda Y, Yamamoto R** (1970) Effect of auxin on β-1→3-glucanase activity in *Avena* coleoptiles. Dev Growth Differ **11:** 287–296
20. **Nevins DJ** (1975) The effect of nojirimycin on plant growth and its implications concerning a role for exo-β-glucanase in auxin-induced cell expansion. Plant Cell Physiol **16:** 347–356
21. **Nevins DJ, Hatfield R, Hoson T, Inouhe M** (1987) Inhibition of elongation by antibodies specific for wall proteins. *In* DJ Cosgrove, DP Knievel, eds, Physiology of Cell Expansion during Plant Growth. American Society of Plant Physiologists, Rockville, MD, pp 122–132
22. **Sakurai N, Masuda Y** (1977) Effect of indole-3-acetic acid on cell wall loosening: changes in mechanical properties and non-cellulosic glucose content of *Avena sativa* coleoptile cell wall. Plant Cell Physiol **18:** 587–594
23. **Sakurai N, Masuda Y** (1978) Auxin-induced changes in barley coleoptile cell wall composition. Plant Cell Physiol **19:** 1217–1223
24. **Sakurai N, Nishitani K, Masuda Y** (1979) Auxin-induced changes in the molecular weight of hemicellulosic polysaccharides of *Avena* coleoptile cell wall. Plant Cell Physiol **20:** 1349–1357
25. **Yamamoto R, Nevins DJ** (1981) Coleoptile growth-inducing capacities of exo-β-(1→3)-glucanases from fungi. Physiol Plant **51:** 118–122
26. **Zarra I, Masuda Y** (1979) Growth and cell wall changes in rice coleoptiles growing under different conditions. II. Auxin-induced growth in coleoptile segments. Plant Cell Physiol **20:** 1125–1133