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Endo-β-Mannanase Activity Present in Cell Wall Extracts of Lettuce Endosperm prior to Radicle Emergence[1]

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Lettuce (*Lactuca sativa* L.) endosperm cell walls isolated prior to radicle emergence underwent autohydrolysis, the rate of which was correlated with whether radicle emergence would subsequently occur. Extraction of endosperm cell walls with 6 M LiCl suppressed autohydrolysis, and the desalted extract possessed activity that was capable of hydrolyzing purified locust bean galactomannan but not arabinogalactan, carboxymethylcellulose, glucomannan, polygalacturonic acid, tomato galactomannan, or native lettuce endosperm cell walls. Some hydrolytic activity was detected on endosperm cell walls if they were modified by partial trifluoroacetic acid hydrolysis or pretreatment with guanidinium thiocyanate. In extended incubations the cell wall enzyme extract released only large molecular mass fragments from locust bean galactomannan, indicating primarily endo-activity. Galactomannan-hydrolyzing activity in the cell wall extract increased as a function of imbibition time and was greatest just prior to radicle emergence. Thermoinhibition (imbibition at 32°C) or treatment with abscisic acid at a temperature optimal for germination (25°C) suppressed both germination and endosperm cell wall mannanase activity, whereas alleviation of thermoinhibition with gibberellic acid was accompanied by significant enhancement of mannanase activity. We conclude that a cell wall-bound endo-β-mannanase is expressed in lettuce endosperm prior to radicle emergence and is regulated by the same conditions that govern germination.

Lettuce (*Lactuca sativa* L.) seed germination is responsive to environmental and hormonal influences, apparently controlled by the thick-walled endosperm tissue that encapsulates the embryo. The presence of the endosperm delays radicle emergence under optimal conditions or prevents it under inhibitory conditions (Weges, 1987; Bradford, 1990). Germination is completed by radicle protrusion through the micropylar endosperm, and the events leading to this process can be inhibited or suppressed by supraoptimal temperatures, water stress, or ABA. Inhibition can be alleviated by exposure to red light, cytokinins, GA, or ethylene and by breaking the integrity of the endosperm (Bewley and Halmer, 1980/1981; Dutta and Bradford, 1994).

Two mechanisms have been proposed to underlie radicle emergence. Embryo expansion due to uptake of water may generate a mechanical force sufficient to rupture the endosperm (Nabors and Lang, 1971a, 1971b); however, thermoinhibition of lettuce seed germination is not due to failure of the embryo to absorb water or to develop sufficient turgor (Weges, 1987; Bradford, 1990; Weges et al., 1991). Alternatively, the restraints imposed by the endosperm may be weakened, possibly through enzyme-mediated degradation (Ikuma and Thimann, 1963).

Detailed structural modifications have been observed specifically in the micropylar region of the lettuce endosperm prior to radicle emergence (Psaras et al., 1981; Georghiou et al., 1983; Psaras and Georghiou, 1983). These modifications did not occur in seeds that were allowed to imbibe under conditions unfavorable for germination. Ikuma and Thimann (1963) suggested that enzyme-mediated endosperm cell wall degradation was responsible for inducing germination in photodormant lettuce seeds based on experiments in which crude preparations of cellulase, pectinase, and pentosanase were introduced under the endosperm envelope. Since lettuce endosperm cell walls are largely composed of polymers of Man (Halmer et al., 1975; Dutta et al., 1994), subsequent studies have focused on mannanases. However, mannan hydrolysis by a soluble endo-β-mannanase could be detected only after radicle emergence had occurred (Halmer et al., 1976; Bewley and Halmer, 1980/1981). To our knowledge, wall-bound enzymes that might be present in the lettuce endosperm have not been extracted or characterized.

We have shown that isolated cell walls prepared from lettuce endosperm tissue prior to radicle emergence are capable of autohydrolysis (Dutta et al., 1994). Rates of autolysis were correlated with the capacity of the seeds to germinate. When walls were prepared from endosperms excised from seeds in which germination was inhibited (thermoinhibited seeds or seeds that were allowed to imbibe in ABA), autolysis rates were suppressed. Conditions favoring germination (for example, incubation of thermoinhibited seeds with GA_3) resulted in enhancement of autolysis rates. Since the cell walls were washed extensively prior to autolysis experiments, the remaining hydrolytic activity was apparently bound tightly to the walls.

The aims of the present study were to extract and characterize the enzyme activity responsible for autohydrolysis in isolated lettuce endosperm cell walls and to determine

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Abbreviations: AG, arabinogalactan; CMC, carboxymethylcellulose; GlM, glucomannan; LBG, locust bean galactomannan; PGA, polygalacturonic acid; TFA, trifluoroacetic acid; TGM, tomato seed galactomannan.

whether a relationship exists between the expression of this activity and the likelihood that radicle emergence will occur.

MATERIALS AND METHODS

Plant Materials and Seed Incubation

Seeds (achenes) of lettuce (*Lactuca sativa* L. cv Pacific; Royal Sluis, Salinas, CA) were placed on two 4.7-cm filter papers in 5-cm Petri dishes moistened with 3.6 mL of distilled water. The dishes were sealed to prevent evaporation and incubated in a controlled temperature chamber in darkness at either 25 or 32°C. For hormone treatment the seeds were allowed to imbibe in 100 μM ABA or GA_3 (Sigma). Unless otherwise stated, enzyme extracts were derived from seeds that had been allowed to imbibe for 12 h.

Wall Preparation and Protein Extraction

After incubation for specific durations, the lettuce seeds were immediately frozen at −80°C. To excise the endosperms frozen seeds were placed on a glass plate over ice and gently pressed with a slight twisting motion at the cotyledon end using the tip of a conical glass rod. The embryo separated from the pericarp and endosperm, and the endosperm typically adhered to the tip of the rod. After the endosperm tissues were blotted with filter paper to remove moisture, they were transferred to microfuge tubes (1.5 mL) and stored at −80°C. Approximately 5500 endosperms (1 g of tissue) were collected for each experiment.

Endosperm tissue was placed in liquid nitrogen and homogenized to a fine powder with a mortar and pestle. The homogenized tissue was transferred to microfuge tubes with 4 mL of cold distilled water and centrifuged at 6000*g* at 4°C for 6 min. The supernatant was removed, dialyzed, and saved as water-soluble extract. The insoluble material was washed five times with ice-cold 50 mM NaCl, five times with cold acetone (−20°C), five times with 50 mM NaCl, and five times with distilled water. Autolytic activity of the isolated walls was determined as described previously (Dutta et al., 1994). The washed walls were extracted by incubation in 3 mL of 1, 2, 3, or 6 M LiCl at 4°C for 12 h. After extraction wall material was collected by centrifugation at 6000*g* and the supernatant was designated as the wall enzyme extract. The extract was dialyzed (Spectra/Por membrane, 29 mm in diameter, 3500 molecular weight cutoff; Spectra/Por, Houston, TX) against 10 mM citrate phosphate buffer, pH 5.0, for 48 h, and the remaining insoluble material was removed by centrifugation. Protein in the LiCl extracts was determined by the Bio-Rad assay using BSA as a standard. Approximately 0.2 μg of protein was extracted per milligram fresh weight of endosperm. Walls prepared from endosperms excised from seeds that were allowed to imbibe under conditions that induce or inhibit germination yielded similar quantities of protein. Cell walls used as a substrate were subjected to 6 M LiCl extraction and then desalted, washed, and boiled for 5 min in distilled water.

Chromatography

A Dionex HPLC system equipped with a TSK-125 column (300 × 7.5 mm; Bio-Rad) was used to resolve the molecular profiles of the LBG substrate (Sigma) before and after treatment with the enzyme preparation. A 400-μL sample was loaded on the column and eluted with sodium phosphate buffer (50 mM, pH 7.2). Fractions (0.5 mL) were collected at a flow rate of 0.3 mL/min and analyzed for sugar content using the phenol-sulfuric acid method (Dubois et al., 1956). The column was calibrated with dextrans (500, 255, and 73 kD) and Glc.

Enzyme and Sugar Assays

Enzyme substrates (0.3%, w/v) were suspended in 150 mM citrate phosphate buffer (pH 5.0) containing 0.02% sodium azide. The enzyme preparation (50 μg of total protein) was incubated with 10 mL of LBG solution purified according to the procedure of McCleary (1978). Enzyme activity was also evaluated in the presence of GIM (gift of Dr. Naoki Sakurai, Hiroshima University, Higashi Hiroshima, Japan), TGM (gift of Dr. Peetambar Dahal, University of California, Davis), and CMC, larch wood AG, and PGA (Sigma). In each case the substrate was incubated with enzyme at 35°C and 1-mL aliquots were removed as a function of time. Total sugars were determined using the phenol-sulfuric acid method (Dubois et al., 1956) and reducing sugar equivalents were determined using the neocuproine assay (Dygert et al., 1965). Enzyme extracts were also administered to lettuce endosperm cell walls. Walls prepared from lettuce endosperm as described above were used directly as substrate, and in some cases walls were modified by mild hydrolysis in 2 N TFA at 25°C for 30 min or extracted with 6 M guanidinium thiocyanate at 25°C for 12 h. TFA treatment removed labile side chains from the polysaccharide, making the main chain more accessible to enzymic attack, and guanidinium thiocyanate has been reported to selectively extract Man-rich polysaccharides (Selvendran et al., 1985). The insoluble material remaining after these treatments was used as substrate. Walls were suspended in buffer to give a concentration of 0.5% (w/v). Commercial enzyme preparations, including purified mannanase (EC 3.2.1.78) from *Aspergillus niger* (Megazyme, New South Wales, Australia), α-galactosidase (EC 3.2.1.22), and Driselase (Sigma; see Fry, 1988), were also evaluated with lettuce endosperm walls as the substrate. Driselase was used to represent a wide spectrum of polysaccharidases known to include exo- and endo-hydrolases capable of hydrolyzing mannans (Fry, 1988). The commercial Driselase preparation was dialyzed in the presence of the incubation buffer and then centrifuged to remove any insoluble precipitate. Fifty micrograms of Driselase protein was used in each incubation mixture.

RESULTS

Inhibition of Autolysis by LiCl Extraction

Radicle emergence from seeds that were allowed to imbibe at 25°C was first observed at about 15 h, and more

than 95% of the seeds completed germination by 17 h. For most studies walls were prepared from lettuce endosperms excised from seeds that had been allowed to imbibe for 12 h at 25°C. Isolated endosperm cell walls exhibited active autolysis at this time, and incubation for 12 h in LiCl suppressed subsequent autolytic rates (Fig. 1). Increasing concentrations of LiCl progressively reduced autolysis, with 6 M LiCl being the most effective. In all cases, LiCl-extracted walls were desalted prior to the initiation of autolysis experiments. Walls without LiCl pretreatment (0 M LiCl) were incubated with distilled water for 12 h and were treated in a manner similar to that of LiCl-treated walls.

Enzymatic Activity of LiCl Extracts

Extracts prepared from lettuce endosperm cell walls using 6 M LiCl were tested for their capacity to hydrolyze LBG, AG, CMC, GlM, PGA, and TGM. Among these substrates, release of reducing sugars was detected only in the presence of LBG (Table I). Activity was abolished by heating the wall extract at 100°C for 5 min. The rate of release of reducing sugars was also a function of the pH of the medium, with maximum activity at pH 5.0 (data not shown). When extracts were incubated with unmodified walls prepared from lettuce endosperm, no release of reducing groups could be detected (Table I). However, some activity was detected using walls modified by partial TFA hydrolysis or by pretreatment with guanidinium thiocyanate; however, this activity was only approximately 10% of that observed with LBG (Table I). Purified endo-β-mannanase from *A. niger* mediated some degradation of the unmodified endosperm walls (0.6% of the substrate hydrolyzed), whereas α-galactosidase had no effect when used alone or in combination with endo-β-mannanase (Table II). The fungal endo-β-mannanase was approximately 10 times more active in the presence of LBG, hydrolyzing approximately 6% of the substrate. Treatment with Driselase resulted in substantial release of reducing groups from the lettuce endosperm walls (Table II).

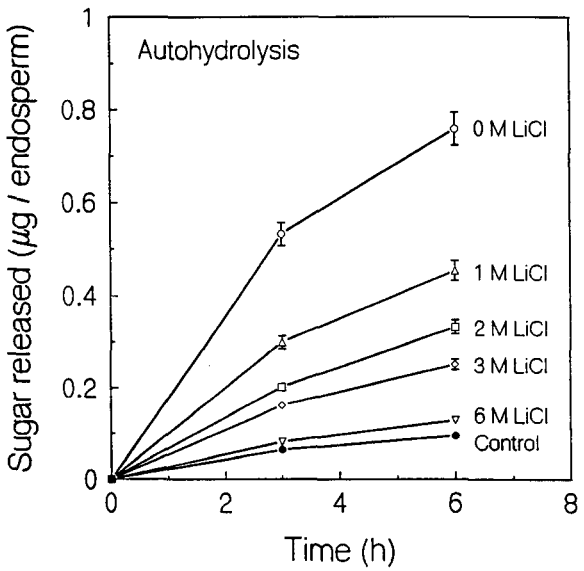

Figure 1. Autohydrolytic release of carbohydrates from isolated lettuce endosperm cell walls incubated in citrate phosphate buffer (150 mM, pH 5.0) as a function of time. Walls were prepared from endosperm tissue excised from seeds that had been allowed to imbibe at 25°C for 12 h and were then treated in LiCl solutions or distilled H_2O (0 M LiCl) for 12 h. After extraction residual endosperm walls were incubated in small columns and product release was measured at the indicated intervals by exchanging the buffer and assaying for total sugars. Fifty endosperms per replicate were used for each treatment and each point represents a mean of five replicates. Vertical bars indicate confidence intervals (P = 0.05) when they exceed the size of the symbol. Control walls were heated in boiling water for 5 min.

Table I. *Activity on various substrates of soluble and cell wall extracts prepared from lettuce endosperm*

Soluble and cell wall extracts were prepared in sequence from lettuce endosperm and incubated with several substrates for 24 h. Reaction products were determined reductometrically. Results are means ± SE of three independent experiments. Numbers in parentheses represent percentages of total substrate hydrolyzed.

Substrate	Enzyme Source	
	Soluble extract	6 M LiCl extract
	μg of Glc-equivalent reducing sugars released	
LBG	374 ± 34 (1.3)	457 ± 26 (1.6)
AG	None	None
CMC	None	None
GlM	None	None
PGA	None	None
TGM	None	None
Lettuce endosperm cell walls	None	None
Modified lettuce endosperm walls[a]	24 ± 6 (0.08)	68 ± 12 (0.2)
Modified lettuce endosperm walls[b]	38 ± 7 (0.13)	46 ± 4 (0.16)

[a] Modified by TFA hydrolysis. [b] Modified by extraction with guanidinium thiocyanate.

Table II. *Activity of commercial enzymes on cell walls prepared from lettuce endosperm*

Cell walls prepared from lettuce endosperm tissue or purified LBG were incubated in pH 5.0 buffer with the enzymes for 24 h. Reaction products were determined reductometrically. Results are means ± SE of three independent experiments. Numbers in parentheses represent percentages of total substrate hydrolyzed.

Enzyme	Substrate	
	Lettuce endosperm walls	LBG
	μg of Glc-equivalent reducing sugars released	
Endo-β-mannanase	177 ± 5 (0.6)	1677 ± 52 (5.6)
α-Galactosidase	None	None
Endo-β-mannanase + α-galactosidase	173 ± 31 (0.6)	1744 ± 92 (5.8)
Driselase	970 ± 66 (3.3)	Not tested

Molecular Weight Profiles of the Substrate and Products

Purified LBG was excluded from the Biosil TSK column as a uniform peak. After the LBG was treated with wall enzyme preparation, partial degradation of the substrate was revealed by changes in the elution profile (Fig. 2). Reaction products were polymeric but reduced in size compared with the original substrate, indicating an endo-activity. The LBG molecular weight profile was not altered by cell wall enzyme preparations from endosperms excised from seeds that had been incubated under conditions not conducive to germination (incubation with ABA or at a thermoinhibitory temperature; see below) (data not shown). Soluble extracts prepared from lettuce endosperm generated smaller molecular weight fragments in addition to the major polymeric peak resulting from wall extracts (Fig. 2).

Relationship between Hydrolyzing Activity and Germination

Cell wall enzyme extracts prepared from endosperms excised from lettuce seeds prior to radicle emergence exhibited LBG-hydrolyzing capacity, with increasing activity as a function of imbibition time (Fig. 3). Cell wall extracts from endosperm tissues excised from seeds that had been allowed to imbibe at a thermoinhibitory temperature (32°C) were much less effective in mediating the release of reducing sugars from LBG compared with extracts from walls of seeds maintained at 25°C, the optimum temperature for germination (Fig. 4). Moreover, seeds that had been allowed to imbibe at a thermoinhibitory temperature (32°C) in water did not germinate, but they did in the presence of 100 μM GA_3. Under these conditions germina-

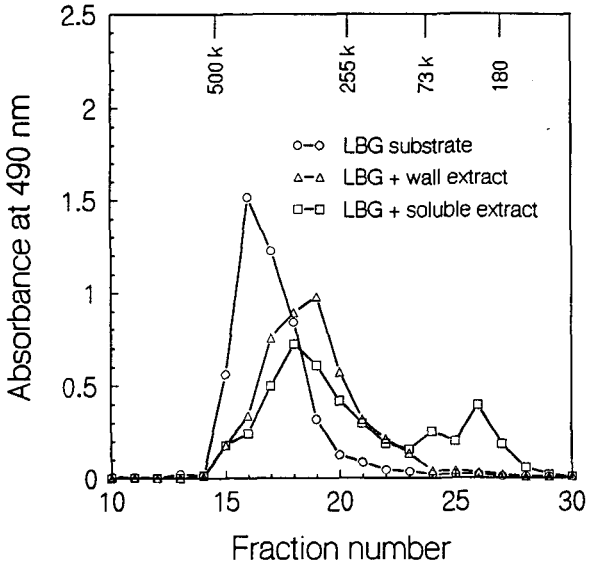

Figure 2. Profile of LBG before and after reaction with soluble and wall extracts prepared from lettuce endosperm. The extracts were reacted with the substrate for 24 h and reaction products were chromatographed on a Biosil TSK-125 column. The fractions where dextran molecular mass standards eluted are indicated on the top axis.

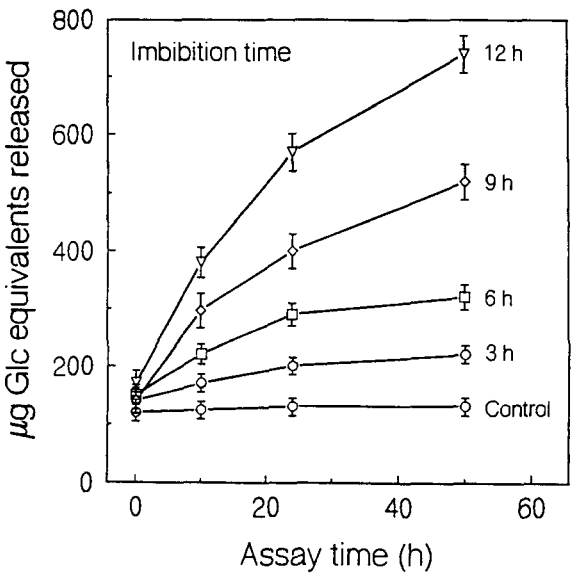

Figure 3. Hydrolysis of LBG by lettuce endosperm cell wall enzyme extracts. Endosperms were excised from lettuce seeds that had been allowed to imbibe for 3, 6, 9, and 12 h at the optimum temperature for germination (25°C), and walls were prepared and extracted with 6 M LiCl. Vertical bars indicate confidence limits (P = 0.05) when they exceed the size of the symbols. Extract (12-h imbibition time) heated in boiling water for 5 min was used as a control.

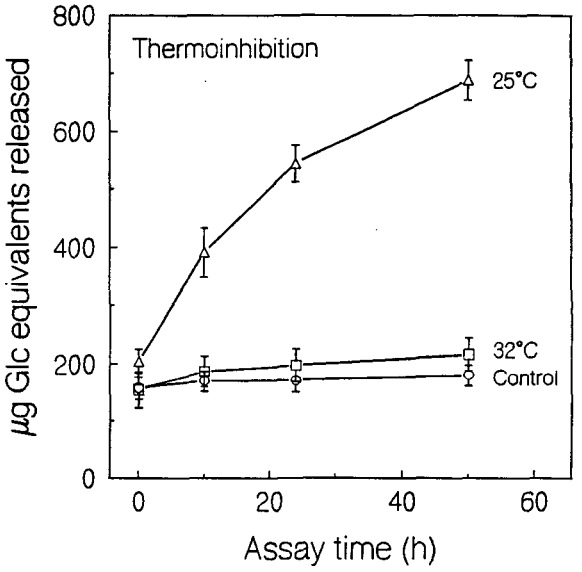

Figure 4. Effect of imbibition temperature on hydrolysis of LBG by lettuce endosperm cell wall enzyme extracts. Endosperms were excised from lettuce seeds that had been allowed to imbibe for 12 h at the temperature optimum for germination (25°C) or at a thermoinhibitory temperature (32°C). Each point represents the mean of three independent experiments. Vertical bars indicate confidence limits (P = 0.05) when they exceed the size of the symbols. Control extracts were heated in boiling water for 5 min.

tion was first observed at about 17 h, and by 20 h all seeds had germinated. Extracts of cell walls prepared from endosperm tissue excised from seeds that had been allowed to imbibe for 14 h at 32°C with or without GA_3 showed that activity measured in the presence of LBG was enhanced by GA_3 (Fig. 5). Seeds incubated with 100 μM ABA at 25°C did not germinate, and endosperm wall extracts prepared from these seeds exhibited significantly lower activity relative to seeds that had been allowed to imbibe in water at 25°C (Fig. 6). The activity in the presence of ABA was comparable with that of the wall extracts prepared from the thermoinhibited seeds and boiled controls (Figs. 4 and 5).

Injection of Enzymes within the Endosperm Envelope

Ikuma and Thimann (1963) reported that application of cell wall hydrolases to induce germination of photodormant lettuce seeds was effective only when the enzymes were injected underneath the endosperm envelope. Since the germination of lettuce seeds is thermoinhibited at supraoptimal temperatures (32°C), we wished to determine whether injection of lettuce mannanases would stimulate germination under those conditions. Soluble and cell wall extracts prepared from lettuce endosperm were injected through the endosperm of thermoinhibited seeds. These attempts to induce germination were consistently inconclusive, however, because the controls (seeds injected with water or boiled extracts) as well as seeds injected with enzyme extracts all completed germination within 12 h.

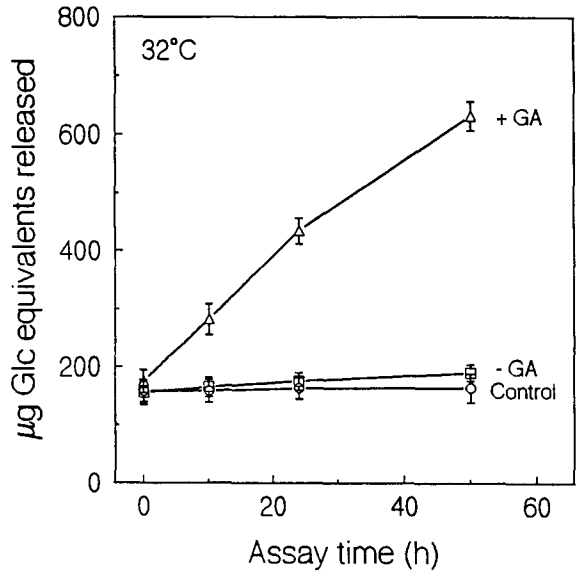

Figure 5. Effect of imbibition temperature and GA_3 on hydrolysis of LBG by lettuce endosperm cell wall enzyme extracts. Endosperms were excised from lettuce seeds incubated for 14 h at 32°C (thermoinhibitory temperature) with (+GA) or without (−GA) 100 μM GA_3. No radicle emergence was observed in the thermoinhibited seeds not supplied with GA_3. Seeds that had been allowed to imbibe in 100 μM GA_3 initiated radicle emergence by 17 h and all seeds in the treatment had completed germination by 20 h. Each point represents the mean of three independent experiments. Vertical bars indicate confidence intervals (P = 0.05) when they exceed the size of the symbols. Control extracts were heated in boiling water for 5 min.

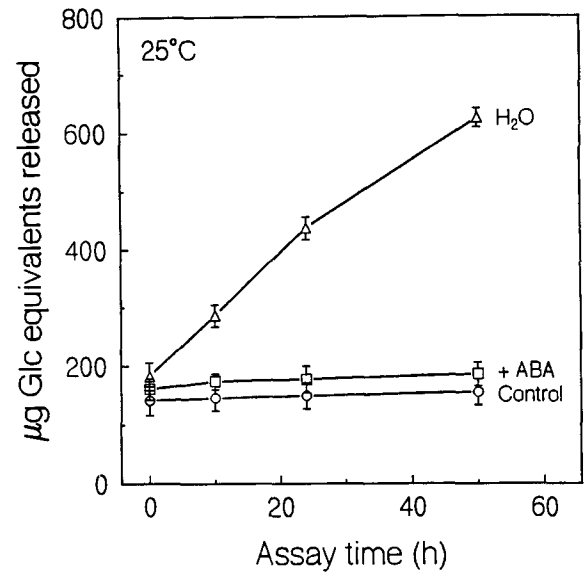

Figure 6. Effect of ABA on hydrolysis of LBG by lettuce endosperm cell wall enzyme extracts. Walls were isolated from seeds that had been allowed to imbibe in 100 μM ABA (+ABA) or in distilled water (H_2O) for 12 h at 25°C. Radicle emergence was not observed with either of the treatments at this time. Seeds that had been allowed to imbibe in ABA for longer periods did not germinate, whereas seeds that had been allowed to imbibe in distilled water initiated radicle emergence at 15 h and all had germinated by 18 h. Each point represents the mean of at least three independent samples. Vertical bars indicate confidence intervals (P = 0.05) when they exceed the size of the symbols. Control extracts were heated in boiling water for 5 min.

Even minor punctures made in the endosperm envelope with the 31-gauge injecting needle completely alleviated thermoinhibition and allowed embryo emergence at the site of puncture (data not shown).

DISCUSSION

Cell walls isolated from lettuce endosperm are capable of autohydrolytic activity, indicating the presence of wall-bound hydrolases (Dutta et al., 1994). In this report we show that mannan-hydrolyzing activity can be extracted from the endosperm walls prior to radicle emergence and that extraction is coupled with a suppression of autolysis (Fig. 1; Table I). At least a component of the extracted activity is attributed to an endo-β-mannanase, based on hydrolysis of purified LBG (β-[1→4]-galactomannan), but no activity toward AG, CMC, GlM, TGM, or PGA (Table I). LBG-hydrolyzing activity in the cell wall extracts of lettuce endosperm exhibited a distinct optimum at pH 5.0, which coincides with the pH response of the walls undergoing autolysis (Dutta et al., 1994).

While the assay measures released reducing groups, size-exclusion chromatography of the products showed that the average polymer size of the LBG substrate was reduced, but monomers were not released, indicating endo-type enzyme activity (Fig. 2). This product profile contrasts with the analysis of products of endosperm cell wall autolysis (Dutta et al., 1994) or of soluble endosperm

extracts (Fig. 2), where both polymeric and monomeric products were released. Because of this fragmentation pattern, endosperm cell wall autolysis was considered to be a process mediated by the activity of endo- and exo-enzymes working in concert (Dutta et al., 1994). The crude extract prepared from the lettuce endosperm wall may contain other hydrolytic activities that were not detected by the range of substrates used here, or other enzymes present in isolated walls may not be extracted or may be inactivated by the extraction conditions. Furthermore, the structure of the lettuce endosperm cell wall is certainly much more complex than that of the purified LBG substrate used here, potentially providing additional sites for enzyme action.

The observed hydrolytic activity of endosperm extracts was relatively low (Table I). Reducing sugars released during the assay corresponded to approximately 2% of the total monomeric fragments that might be potentially available if all of the glycosidic linkages were cleaved. However, the low enzyme activity represented by the cell wall extracts is consistent with the autolytic degradation of lettuce endosperm cell walls, in which approximately 2% of the wall material was released (Dutta et al., 1994). The extent to which autolysis occurs varies, depending on the origin of the tissue. In walls prepared from maize (*Zea mays* L.) coleoptile (Huber and Nevins, 1979) and azuki bean (*Vigna angularis* [Willd.] Ohwi and Ohasih) epicotyl (Hoson, 1990), as much as 10% of the wall material was solubilized and virtually all of the target polysaccharide was released during autolysis. In lettuce, the low activity may suggest the specific nature of the enzyme(s) present in the extracts as well as characteristics of the substrate. It is likely that the limited number and specific nature of the bonds susceptible to catalysis during autolysis within lettuce endosperm cell walls may restrict activity. The conditions imposed by extraction of the wall might also unfavorably alter its structure, rendering it less susceptible to hydrolysis even in the presence of constitutive enzymes. The susceptibility of the wall substrate to enzyme action was enhanced by mild acid hydrolysis and treatment with guanidinium thiocyanate (Table II). Although treatment of endosperm cell walls with high salt concentrations effectively inactivated autolysis, and the soluble extract possessed polysaccharide hydrolyase activity, we were unable to reconstitute autolysis by combining the extract with inactive walls. The limited degradation of endosperm cell walls when supplied with purified endo-β-mannanase preparation (Table II) is consistent with the idea that a limited number of specific hydrolytic sites are available for enzyme action. Structural details of matrix polysaccharides and the associated linkages between the polymers that constitute endosperm walls are not known.

Previous investigations using soluble extracts found that endosperm mannanase activity developed only after radicle emergence (Bewley and Halmer, 1980/1981; Dulson and Bewley, 1989; Halmer, 1989). However, cell wall endo-β-mannanase activity described here is clearly present prior to radicle emergence (Table I) but is tightly bound to the cell walls and likely would not have been included in soluble extracts characterized previously. The wall-bound and soluble activities may represent different isozymes, since multiple isoforms of endo-β-mannanase have been reported in many seeds, including lettuce (Dulson and Bewley, 1989; Dirk et al., 1995; Nonogaki et al., 1995). In contrast to previous reports, we found that soluble extracts prepared from endosperms excised from seeds prior to radicle emergence exhibited LBG-hydrolyzing activity (Table I). The amount of tissue per sample in our investigations was considerably larger than that used previously (approximately 6000 endosperms versus 200). Because of the larger sample size, endo-β-mannanase that might be restricted to only the micropylar endosperm region prior to radicle emergence, as is the case in tomato (*Lycopersicon esculentum* Mill.; Nonogaki and Morohashi, 1996), may have been detectable in our assays, even though the assay itself (reducing sugar release) is less sensitive than the viscometric assay used by others. Lettuce cultivars differ in endo-β-mannanase abundance and isozyme complements (Dirk et al., 1995); however, when we used our methods to prepare extracts from cv Grand Rapids (used in other studies), results similar to those presented here for cv Pacific were observed (data not shown). In any case, our study has demonstrated a cell wall-bound endo-β-mannanase activity that, to our knowledge, has not been reported previously and is present prior to radicle emergence.

Autolytic activity of cell walls prepared from the endosperm tissue of lettuce seeds is correlated with germination; conditions favoring or suppressing germination yield walls with higher or lower rates of autolysis (Dutta et al., 1994). Similarly, endo-β-mannanase activity can be extracted from endosperm cell walls of lettuce seeds that were allowed to imbibe under conditions conducive for germination (Fig. 3). Incubation of seeds at thermoinhibitory temperatures results in total prevention of germination and almost complete suppression of endo-β-mannanase activity (Fig. 4). When thermoinhibited seeds were allowed to imbibe in GA_3 solution, germination was restored and a corresponding increase in endo-β-mannanase activity was observed (Fig. 5). Inhibition of germination by ABA also suppressed the activity of endo-β-mannanase in extracts of the cell walls of lettuce endosperm (Fig. 5), and soluble mannanase activity in isolated endosperms is also regulated by ABA (Dulson et al., 1988). The activity of extracted cell wall endo-β-mannanase therefore correlates well with autolysis and with germination behavior of lettuce seeds.

Unfortunately, we were unable to duplicate the results of Ikuma and Thimann (1963) by injecting the cell wall extracts underneath the endosperm envelopes of thermoinhibited seeds. We found that responses of thermoinhibited seeds to injected enzymes could not be evaluated because all seeds subjected to penetration by the needle germinated. Although Ikuma and Thimann (1963) used photodormant instead of thermodormant seeds, we observed similar alleviation of dark inhibition of lettuce germination when the endosperm was cut or punctured. It should be noted that even in their experiments approximately 50% of the control (water-injected) seeds also germinated.

Although our results do not conclusively demonstrate a mechanistic connection between the activity of lettuce endosperm cell wall endo-β-mannanase and germination, autolysis of endosperm cell walls causes wall degradation and this process is inactivated by extraction with LiCl. Moreover, the LiCl extract possesses activity that is responsive to the same environmental or hormonal conditions that induce or inhibit germination. Based on the high mannan content of the endosperm cell walls, the demonstrated activity of the enzyme on purified galactomannan substrate, the presence of activity prior to radicle emergence, and the close correlation between enzyme activity and the completion of germination, we suggest that this enzyme is likely to be involved in the disassembly of endosperm cell walls associated with radicle emergence.

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