Plant Physiol. (1900) 94, 411–416
0032-0889/90/94/0411/06/$01.00/0

Received for publication December 29, 1989
Accepted March 23, 1990

Cell Wall and Enzyme Changes during the Graviresponse of the Leaf-Sheath Pulvinus of Oat (*Avena sativa*)[1, 2]

David M. Gibeaut, Nadarajah Karuppiah, S.-R. Chang, Thomas G. Brock*[3], Babu Vadlamudi, Donghern Kim, Najati S. Ghosheh, David L. Rayle, Nicholas C. Carpita, and Peter B. Kaufman

Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907 (D.M.G., N.C.C.); Department of Biology, University of Michigan, Ann Arbor, Michigan 48109 (N.K., S.-R.C., T.G.B., B.V., D.K., P.B.K.); Department of Biology, Eastern Michigan University, Ypsilanti, Michigan 48197 (N.S.G.); and Department of Biology and The Molecular Biology Institute, San Diego State University, San Diego, California 92182 (D.L.R.)

ABSTRACT

The graviresponse of the leaf-sheath pulvinus of oat (*Avena sativa*) involves an asymmetric growth response accompanied by several asymmetric processes, including degradation of starch and cell wall synthesis. To understand further the cellular and biochemical events associated with the graviresponse, changes in cell walls and their constituents and the activities of related enzymes were investigated in excised pulvini. Asymmetric increases in dry weight with relatively symmetric increases in wall weight accompanied the graviresponse. Starch degradation could not account for increases in wall weight. However, a strong asymmetry in invertase activity indicated that hydrolysis of exogenous sucrose could contribute significantly to the increases in wall and dry weights. Most cell wall components increased proportionately during the graviresponse. However, β-D-glucan did not increase symmetrically, but rather increased in proportion in lower halves of gravistimulated pulvini. This change resulted from an increase in glucan synthase activity in lower halves. The asymmetry of β-D-glucan content arose too slowly to account for initiation of the graviresponse. A similar pattern in change in wall extensibility was also observed. Since β-D-glucan was the only wall component to change, it is hypothesized that this change is the basis for the change in wall extensibility. Since wall extensibility changed too slowly to account for growth initiation, it is postulated that asymmetric changes in osmotic solutes act as the driving factor for growth promotion in the graviresponse, while wall extensibility acts as a limiting factor during growth.

The graviresponse of the leaf-sheath pulvinus of oat is initiated by the sedimentation of starch grains, which culminates in a hormone-mediated growth response (7, 22). During the growth response, the starch grains are hydrolyzed in an asymmetric manner (28). The growth response involves not only cell expansion but also the synthesis of new wall materials (7, 14). Cell division is not involved (14).

During cell expansion in cereal grasses (such as oat), a mixed linkage β-D-glucan is synthesized (8). The basic structure is repeating units of cellotriosyl and cellotetraosyl units connected by a single (1→3)β-D-glucosyl linkage (21, 29). β-D-Glucan of developing maize seedlings contains additional substructure that permits hydrolysis by a specific *endo*-β-D-glucanohydrolase at regularly spaced sites about every 50 glucosyl residues (20). This *endo*-β-D-glucanohydrolase and an *exo*-β-D-glucohydrolase, which specifically hydrolyze β-D-glucan, have been partially purified from cell walls of maize and were implicated in the turnover of glucan (19, 20). Gibberellic acid markedly reduces the proportion of β-D-glucan in growth-stimulated leaves of dwarf maize and increases *endo*-β-D-glucanohydrolase activity concomitantly (10). Auxin accelerates the loss of β-D-glucan from walls of excised coleoptile tisues deprived of exogenous sugar (24), but there is no such decrease in glucan content in intact tissues during maximal growth (8) or in excised tissues given sucrose (13). Both auxin and gibberellins act in the graviresponse of the oat pulvinus (4, 7, 14), indicating that hormone-induced changes in β-D-glucan content may accompany the graviresponse.

In this study, we report changes in the cell wall that are associated with the graviresponse of the leaf-sheath pulvinus of oat (*Avena sativa*). We also report changes in the activities of enzymes related to carbon utilization associated with wall synthesis. Our specific interests include (a) the possible role of starch as a source of substrate for wall materials, (b) those changes in cell wall constituents that occur during the graviresponse, and (c) those changes in wall constituents and enzyme activities that may act specifically in growth regulation.

MATERIALS AND METHODS

Materials

Oat plants (*Avena sativa* L. cv Victory, from Svenska Allmanna A.B., Svalöf, Sweden) were raised from seed in a greenhouse with supplemental lighting to maintain a

[1] This article is dedicated to the memory of Dr. Ruth Satter, who made landmark contributions to the physiology of leaf movement and the pulvinus response in plants. Journal Paper 12,474 of the Purdue University Agriculture Experiment Station.

[2] Supported by National Aeronautics and Space Administration (NASA) grants NAGW-1849 (D.L.R.) and NAGW-1600 (P.B.K.) and U.S. Department of Energy DE-FG02-88-ER-13903 (N.C.C.), as well as a NASA Research Associate Award to T.G.B.

[3] Current address: Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109-0618.

light:dark ratio of 18:6 h (6). After 4 weeks, plants were transferred to a growth chamber with the same light:dark ratio with constant temperature at 25 ± 1°C until plants were approximately 45 d old. For experimentation, plants were selected for length of next-to-last (p-1 locus) internode greater than 4 cm, at which stage the next-to-last (p-1) leaf-sheath pulvinus was maximally competent to show a gravi-response (6). Gravistimulation involved placing stem segments, cut 3 cm below and 5 cm above the pulvinus, horizontally between paper towelling and glass plates, with 0.1 M sucrose supplied to the segment bases through the towelling. Similarly prepared segments left in a vertical orientation were used for comparison.

Cell Wall Analysis

After treatment, the pulvini were excised from the remaining internodal tissue and divided into upper and lower halves. Pulvinus tissue was frozen on Dry Ice upon excision, stored at −80°C until all treatments were completed, then lyophilized and weighed before analysis. The dried tissues (50–200 mg) were homogenized with a glass-glass homogenizer in 3 mL of cold buffer, 50 mM Tes-KOH, pH 7.2, containing 30 mM ascorbic acid, and the homogenizer was washed twice with 3 mL of buffer. The homogenates and each of the subsequent washes (10 mL) were centrifuged for 10 min at 1000*g*. The pellets were washed twice with 0.5 M KH_2PO_4 buffer (pH 7.0) and twice in distilled water. Lipids were extracted once in chloroform:methanol (1:2), and once in chloroform:methanol (1:1). Each extraction was heated to 45 to 50°C for 30 min. The pellets were washed once in methanol and twice in distilled water. Starch was extracted from the pellets in 10 mL of DMSO stirred for 24 h. The pellets were washed twice in distilled water before freeze drying.

Starch from the DMSO extract was quantified according to Carpita and Kanabus (9). Glucose released by digestion of the starch with glucoamylase (1,4-α-D-glucohydrolase (EC 3.2.1.3) from *Rhizopus niveus*; Miles Laboratories) was measured by a coupled hexokinase and glucose-6-phosphate dehydrogenase assay. The efficiency of the starch hydrolysis was determined by digestion of amylopectin, and the glucose analysis was quantified by comparison with D-glucose standards.

Protein was assayed according to Bradford (3) (commercial reagent from Pierce) with BSA as standard. Total sugars were assayed by a phenol-sulfuric acid method (16) with glucose and xylose as standards. Uronic acids were assayed by a carbazole method (15), modified by addition of sulfamate to reduce interference from neutral sugars (17), with glucuronic and galacturonic acids as standards.

Hemicellulose materials were hydrolyzed in 1 mL of 2 M TFA containing 1 μmol *myo*-inositol (internal standard) for 90 min at 120°C, and the TFA was evaporated in a stream of nitrogen gas. The distribution of neutral sugars was determined using gas-liquid chromatography after reduction with $NaBH_4$ and preparation of alditol acetates according to Blakeney *et al.* (2) as modified by Carpita and Shea (11).

Partially methylated alditol acetate derivatives were also prepared using *n*-butyllithium anion and methyl iodide (23). The per-*O*-methylated polymers were recovered with Sep-Pak C_{18} cartridges (Waters; purchased from Millipore), as described by Carpita and Shea (11). The polymers were hydrolyzed with TFA and the sugars were reduced with $NaBH_4$ as described above. GLC-electron impact MS of these derivatives was performed as described by Carpita and Shea (11).

Based on the proportions determined using glycosidic linkage analysis from the per-*O*-methylated alditol acetate derivatives and the quantitation of neutral sugars from the alditol acetate derivatives, we estimated the proportions of the major cell wall polysaccharides as described by Bacic *et al.* (1) and Carpita (8). The principle noncellulosic polymers were β-D-glucan, arabinan, xyloglucan, and glucuronoarabinoxylan. β-D-Glucan was the sum of 3-linked glucosyl units and a proportion of the 4-linked glucosyl units three times that of 3-linked units, a factor based on the known fine structure of the glucan (21, 29). Arabinan was the total amount of 5-arabinose. Xyloglucan was the sum of *t*-xylosyl, 4,6-glucosyl, and a proportion of the 4-glucosyl units one-half that of the 4,6-glucosyl units (1, 8). Glucuronoarabinoxylan was determined from the proportion of 4-, 2,4- and 3,4-xylosyl units, and the *t*-arabinofuranosyl units corresponding to the xylosyl branch residues (1, 8). The remaining 4-linked glucosyl units and any unhydrolyzed cell wall material were considered to be cellulose. No cell wall material remained after TFA hydrolysis of the per-*O*-methylated cell walls except for about 10% of the cell wall of the gravistimulated bottom half of the pulvinus.

Alternatively, the amounts of mixed linkage β-D-glucan were estimated by enzymic digestion with an *endo*-β-D-glucanohydrolase from *Bacillus subtilis*. The enzyme purification and assay procedures were performed as described by Carpita and Kanabus (10). Two to 5 mg of cell walls were suspended in 2 mL of 0.05 M sodium phosphate-citrate buffer (pH 6.0) and 50 μL of the enzyme preparation (1.5 μg protein) was added. Samples were incubated at 40°C with a drop of toluene in each sample to inhibit bacterial growth. After 24 h, the suspensions were centrifuged to sediment unhydrolyzed material and 200 μL of the supernatant was withdrawn for determination of reducing sugars (27). Because this enzyme cleaves β-D-glucan into about equal amounts of cellotriosyl- and cellobiosyl-(1,3)-β-D-glucose (21, 25, 29), the amounts of β-D-glucan were estimated from reducing equivalents × 3.5, using D-glucose as a standard.

Invertase Assay

Pulvinus halves were homogenized in 1:5 ratio 10 mM sodium phosphate buffer (pH 6.0) containing 1 mM DTT, 1 mM EDTA, 0.1 mM PMSF, and 1 mM benzamide in a mortar. The homogenate was spun at 30,000*g* for 20 min and the supernatant obtained was assayed for invertase activity. This enzymatic reaction was carried out in a total volume of 0.5 mL containing 50 mM sodium acetate buffer (pH 5.5). The reaction mixture was incubated for 30 min at 30°C and terminated by the addition of Somogyi reagent (26). The resulting reducing sugars were detected by Nelson reagent (26) and quantitated by measurement of *A* at 550 nm.

Table I. *Composition of Pulvinus Halves (Gross Components) by Weight, with and without Gravistimulation*

Treatments were for 48 h at 30°C with segment bases in 0.1 M sucrose. Values are average values using 50 pulvinus halves. Values in parentheses are coefficients of variation.

Treatment	Dry Weight	Cell Wall Weight	Wall Weight / Dry Weight	Starch Weight
	mg			*μg*
Vertical	140	58.2	0.409	690 (29.7)
Horizontal, upper half	190	80.7	0.425	653 (1.27)
Horizontal, lower half	262	85.3	0.326	393 (9.08)

Glucan Synthase Assay

Microsomes were extracted from homogenized tissue in a buffer of 250 mM sucrose, 3 mM EDTA, 0.5% (w/v) PVP-40 and 70 mM Tris-HCl (pH 7.0). The extract was centrifuged at 10,000*g* for 20 min and the supernatant was used for the glucan synthase assays. Assays were conducted in a 100 μL mixture containing 1 mM UDP-[^{14}C] glucose (0.1 mCi/mmol), 5 mM $MgCl_2$, 5 mM cellobiose, and 50 mM Tris-HCl (pH 7.0). The level of protein used was about 500 μg per assay. The mixture was incubated at 25°C for 10 min. Each reaction mixture was spotted on a Whatman GF/A filter disk, dried and placed in a culture tube, then washed successively with 10 mL 66% (w/v) ethanol containing 0.85 mM EDTA, 10 mL 66% ethanol, and 10 mL 70% ethanol. The filter papers were rinsed with acetone and were air-dried, and radioactivity was measured by scintillation counter.

Cell Wall Extensibility Analysis

The cell wall extensibility of isolated pulvini was analyzed by the Instron method (12). Briefly, at intervals after gravistimulation, pulvini halves were isolated from stem segments and boiled in methanol for 5 min. After rehydration in water, samples were subjected to stress/strain analysis using an Instron extensometer and an applied load of 100 g. Presented values are for total cell wall extensibility and are considered to approximate the plastic extensibility component, as the elastic extensibility was found to be negligible (data not presented).

Statistical Analysis

Statistical significance was evaluated using the Student's *t* test, using a level of confidence of 0.05.

RESULTS

During gravistimulation, the oat pulvinus increased significantly in dry weight, in both upper and lower halves (Table I). This response was asymmetric, with the greatest change being in the lower halves. The cell wall weight also greatly increased following gravistimulation, indicating synthesis of new wall material. This change was not strongly asymmetric. The proportion of the dry weight attributable to cell wall weight in upper halves of gravistimulated pulvini was comparable to that in vertical pulvini. However, this proportion was significantly reduced in lower halves, indicating that much of the increase in dry weight was through synthesis of non-wall materials. Starch was asymmetrically degraded, with the greatest loss being in the lower halves. However, the total weight of degraded starch was much less than the increase in cell wall weight following gravistimulation. This indicated that starch could be only a minor source of carbon for wall synthesis.

Sucrose was provided as an exogenous carbon source in all experiments. Invertase, which hydrolyzes sucrose to produce D-glucose and D-fructose, displayed a statistically significant asymmetry of activity within 3 h after gravistimulation (Table II). This asymmetry increased by 6 h and persisted through 48 h after gravistimulation. This indicated that sucrose was hydrolyzed in an asymmetric pattern in gravistimulated pulvini. The changes in invertase activity resulted from large increases from pretreatment levels: by 24 h, the invertase activity in lower halves had increased 28-fold over initial levels; in upper halves, the increase was 7-fold (data not shown).

It was assumed that a significant amount of the carbon from sucrose would be used to contribute to the observed increase in cell wall material during the graviresponse. What changes in cell wall materials were associated with the graviresponse? Analysis of constituents of cell walls from vertical pulvini indicated that arabinoxylan and cellulose were the major components, comprising over 70% of all materials by weight (Fig. 1). Additional constituents of the ungravistimulated pulvinus wall included xyloglucan (12%), uronic acid (7%), arabinan (4%), protein (4%), and β-D-glucan (2%). Following gravistimulation, all components increased proportionately (data not shown), except β-D-glucan (Fig. 2). By two separate analytical techniques, β-D-glucan was found to increase significantly in lower halves of gravistimulated pulvini. No statistically significant change was observed in upper halves, relative to ungravistimulated pulvini. The ratio of β-D-glucan content, lower halves to upper halves, was on the order of 3:2. By the β-D-glucanase technique, the asymmetry of β-D-glucan was detectable only after 6 h (Fig. 3). The asymmetry increased as the graviresponse continued and persisted through 48 h.

Glucan synthase activity, measured as incorporation of label from UDP-[^{14}C] glucose, was found to increase significantly in crude membrane fractions isolated from lower halves

Table II. *Comparative Distribution of Extractable Invertase Activity in Upper versus Lower Halves of Gravistimulated Oat Pulvini*

Time after Gravistimulation	Invertase Activity	
	Upper half	Lower half
h	%	
Initial	55.6	44.4
3	33.5	66.5
6	19.0	81.0
12	25.0	75.0
48	24.8	74.2

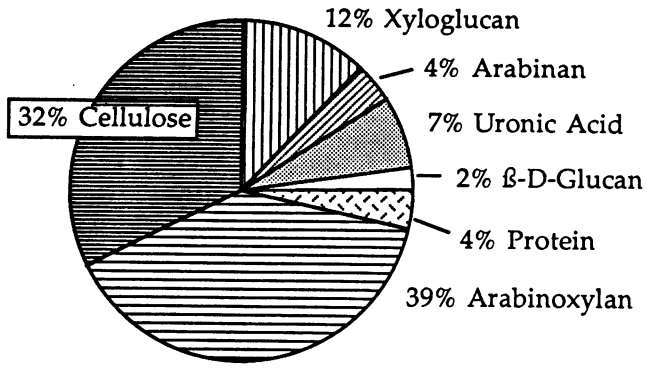

Figure 1. Composition of the cell wall of an ungravistimulated oat pulvinus.

of pulvini that had been gravistimulated (Fig. 4). The glucan synthase activity was also found to increase in upper halves over this interval, but this change was not statistically significant at the 0.05 level of confidence. The increase in glucan synthase activity in lower halves did not occur before 6 h (data not presented). In preliminary tests using the membrane fractionation protocol of Gibeaut and Carpita (18), the change in glucan synthase activity was identified as being limited to the plasmalemma (data not presented).

Only modest changes in major noncellulosic sugars were found during the graviresponse (Fig. 5). The mol% of arabinose increased slightly in both upper and lower halves with a proportional decrease in the proportions of xylose. In contrast, the content of noncellulosic glucose in the walls of the lower halves rose slowly from about 10 to 12 mol%, whereas the mol fraction of glucose in the upper halves decreased slightly. This produced a very modest asymmetry of glucose as the graviresponse proceeded.

The overall cell wall extensibility of isolated pulvinus halves, as measured by the Instron technique, also changed slowly over time (Fig. 6). The first statistically significant change did not occur until after 2 h. There was a clear asymmetry in this change, with the greatest increase in plastic extensibility occurring in lower halves. This increase in extensibility of lower halves continued over 24 h and persisted through 48 h. Interestingly, the total extensibility of upper halves of gravistimulated pulvini also increased.

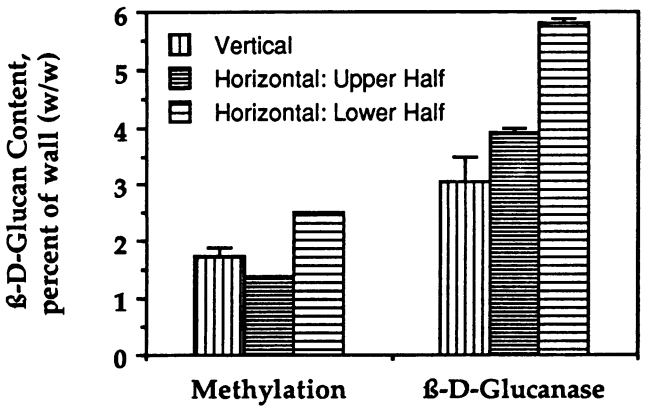

Figure 2. Distribution of β-D-glucan in pulvinus halves, with or without gravistimulation. The β-D-glucan content was determined by two separate techniques: a methylation method and a β-D-glucanase method. Results are presented as the percent of total wall weight. *Bars* indicate SE.

DISCUSSION

The graviresponse of the leaf-sheath pulvinus of oat is a hormone-mediated growth response, with growth being promoted increasingly from uppermost point to lowermost (*cf.* 6, 7). As documented here, there is a significant amount of synthesis of wall material associated with this response (Table I). Starch grains, which apparently play a pivotal role in graviperception (5, 28), are also degraded during the ensuing growth (28). Their contribution to wall synthesis, however, must be minor, considering the magnitude of cell wall synthesis. Exogenous sucrose, in contrast, apparently contributes significantly to the growth response. This is reflected in both the difference in magnitude of gravitropic response observed without sucrose (14, 22) and the strong asymmetry in invertase activity (Table II). It should be noted that additional wall-bound isozymes of invertase occur in the oat pulvinus and that these appear to increase in activity in lower halves following gravistimulation (N. Karuppiah, P. B. Kaufman, unpublished results).

It is striking that the increase in cell wall dry weight in upper halves is close to that in lower halves (Table I). It is also remarkable that changes in most major wall constituents

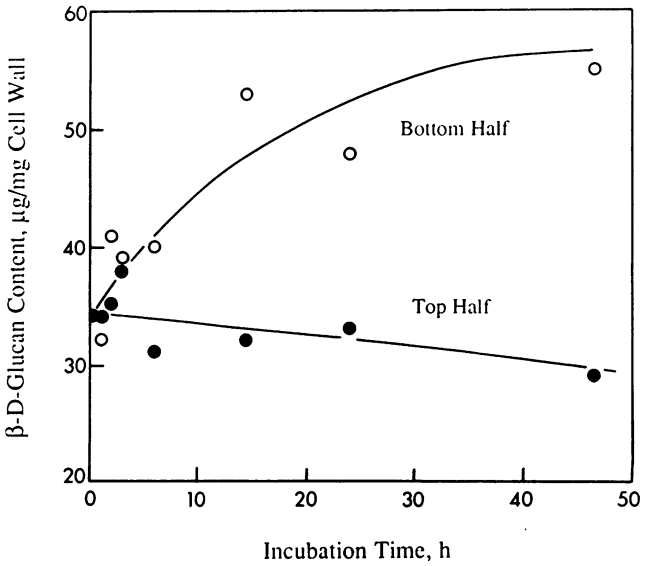

Figure 3. Kinetics of change in β-D-glucan during gravitropism. β-D-Glucan was hydrolyzed with a mixed-linkage-specific *Bacillus subtilis endo* (1→4)β-D-glucanohydrolase that yields about equal amounts of cellobiosyl- and cellotriosyl-(1→3)β-D-glucose as products. Hence, the β-D-glucan content was calculated from reducing equivalents × 3.5 glucosyl units/reducing equivalent. Values are the mean of two determinations of the top halves (closed symbols) and bottom halves (open symbols) of the horizontally oriented pulvini. *Bars* indicate SE.

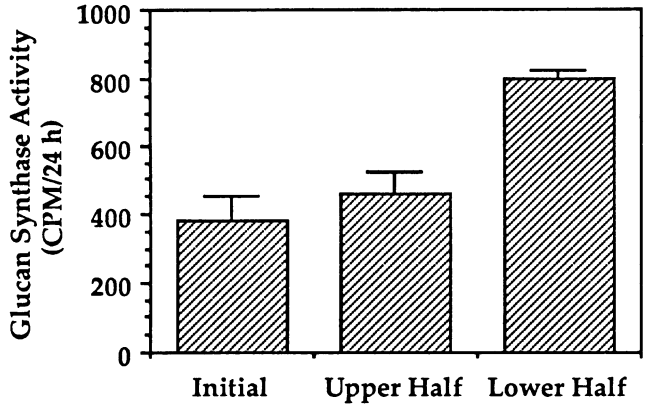

Figure 4. Changes in glucan synthase activity associated with the graviresponse. Glucan synthase activity was assayed as the incorporation of UDP-[^{14}C]glucose into crude membrane fractions from pulvinus tissue. Assays were performed on tissue from upper and lower halves of pulvini gravistimulated for 24 h, or from pulvinus halves of untreated tissue. *Bars* indicate SE.

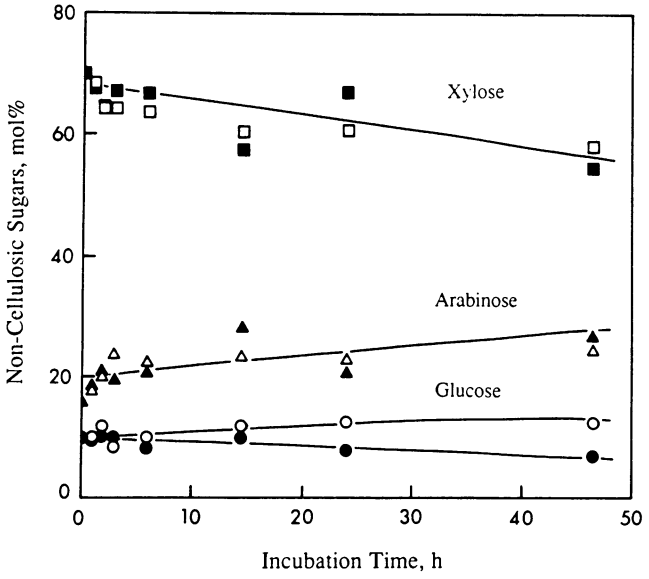

Figure 5. Comparison of changes in composition of the major cell wall noncellulosic sugars during the gravitropic growth response of the oat pulvinus. Excised pulvini were oriented horizontally and incubated for up to 48 h. Cell walls were hydrolyzed in 2 M TFA and alditol acetate derivatives prepared and separated by GLC. *Open symbols* are sugars from lower halves of pulvini; *closed symbols* are from upper halves. Values are mol% after calculation of response factors compared to a *myo*-inositol internal standard.

are approximately parallel in upper and lower halves. These points are particularly of interest when it is noted that there is a striking asymmetry in pulvinus dry weight. This indicates that there must be a significant asymmetry in non-wall materials, such as lipids, proteins and soluble carbohydrates. Considering the asymmetry in invertase activity, it is possible that much of the dry weight increase reflects accumulation of soluble carbohydrates. Such an accumulation could drive growth through depression of intracellular osmotic potential. The asymmetry in glucose may simply reflect the asymmetry in invertase activity.

The only major asymmetric change in a wall component was in β-D-glucan. Still, this asymmetry was relatively small. Furthermore, it appeared to be too slow to be associated with growth initiation; the graviresponse typically begins in less than an hour (14), whereas no significant asymmetry in β-D-glucan was observed by 2 h (Fig. 3). Alternatively, the assay may have been too insensitive to measure the earliest changes. Consistent with this possibility is the correlation between glucan content and graviresponse; β-D-glucan content continued to climb over 48 h in lower pulvinus halves, and the graviresponse will continue to occur well past that time point (5). That is, the pattern of change in β-D-glucan content roughly parallels the pattern of change in angle in the graviresponding pulvinus. The change in β-D-glucan content appears to result, at least in part, from increased glucan synthase activity.

Minor, slow changes in non-cellulosic sugars are associated with the graviresponse. The assay of β-D-glucan content by specific hydrolysis via the *B. subtilis* glucanase (Fig. 2) indicated that the small increases in noncellulosic glucose (Fig. 5) were a result of the increase in β-D-glucan in the lower halves. The glucanase releases mostly tri- and tetrasaccharides from oat bran β-D-glucan, but only about 70% of the glucan is hydrolyzed and the remaining unhydrolyzed material precipitates from solution (data not shown). Nevins and colleagues (18, 19) showed that, in other regions of the seedling, β-D-glucan contains larger stretches of (1→4)β-D-glucosyl linkages that are resistant to hydrolysis by the *B. subtilis* enzyme. Hence, values for β-D-glucan determined enzymically will be

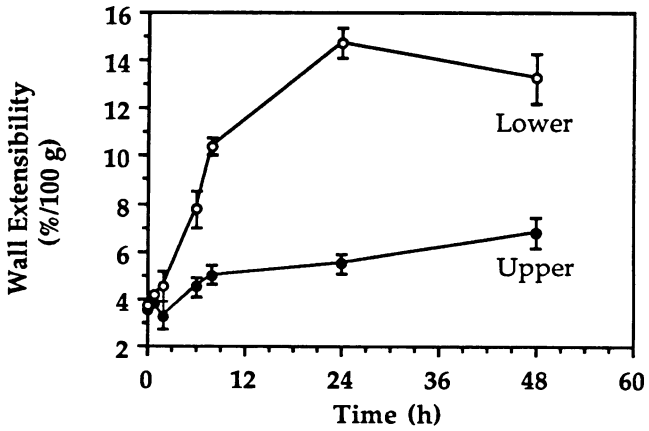

Figure 6. Kinetics of change in cell wall extensibility of upper and lower halves of gravistimulated pulvini.

underestimated slightly. Nevertheless, the differences in content between upper and lower halves are not a result of accessibility to the β-D-glucan by the enzyme in whole-wall preparations. Noncellulosic glucose is increased in acid hydrolysates and preextraction of the hemicelluloses with KOH did not alter values obtained by enzymatic digestion.

The asymmetric change in cell wall extensibility (Fig. 6) indicates a role for changing wall properties in the graviresponse. However, as was the case for β-D-glucan, the kinetics of change are too slow to indicate a crucial role in response initiation. Like β-D-glucan, total extensibility of lower halves increased with time, and the asymmetry persisted through 48 h. Since β-D-glucan was the only cell wall constituent found to change significantly during the graviresponse, it seems likely that β-D-glucan plays an important role in determining wall extensibility.

The graviresponse of the oat pulvinus is a growth response, which includes not only cell elongation but also a 60% increase in total pulvinus dry weight and a 40% increase in total pulvinus wall weight over 48 h (Table I). This indicates that cell elongation and wall loosening are accompanied by wall synthesis and solute accumulation. Given the relatively large increases and asymmetry in invertase activity, and the apparent large accumulation of solutes, we hypothesize that, in the leaf-sheath pulvinus, an osmotic potential gradient acts as the driving factor for growth. In contrast, given the relatively slow and small changes in cell wall components and wall extensibility, we hypothesize that wall extensibility is the limiting factor in pulvinus growth.

ACKNOWLEDGMENTS

The technical support of Jennifer Kyler and Anna Olek is gratefully acknowledged. Thanks also to Carol M. Fast for her support and invaluable contributions, and to Peter Thomas and Allison Elizabeth for technical assistance.

LITERATURE CITED

1. **Bacic A, Harris PJ, Stone BA** (1988) Structure and function of plant cell walls. *In* J Preiss, ed, The Biochemistry of Plants, Vol 14. Academic Press, New York, pp 297–372
2. **Blakeney AB, Harris PJ, Henry RJ, Stone BA** (1983) A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydr Res **113:** 291–299
3. **Bradford MM** (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254
4. **Brock TG, Kaufman PB** (1988) Altered growth response to exogenous auxin and gibberellic acid by gravistimulation in pulvini of *Avena sativa*. Plant Physiol **87:** 130–133
5. **Brock TG, Kaufman PB** (1988) Competency for graviresponse in the leaf-sheath pulvinus of *Avena sativa:* onset to loss. Am J Bot **75:** 1672–1677
6. **Brock TG, Lu CR, Ghosheh, NS, Kaufman PB** (1989) Localization and pattern of graviresponse across the pulvinus of barley (*Hordeum vulgare*). Plant Physiol **91:** 744–748
7. **Brock TG, Kaufman PB** (1990) Movement in grass shoots. *In* RL Satter, H Gorton, T Vogelmann, eds, The Pulvinus: Motor Organ for Leaf Movement. American Society of Plant Physiologists, Rockville, MD, pp 59–71
8. **Carpita NC** (1984) Cell wall development in maize coleoptiles. Plant Physiol **76:** 205–212
9. **Carpita NC, Kanabus J** (1987) Extraction of starch with dimethylsulfoxide and quantitation by enzymic assay. Anal Biochem **161:** 132–139
10. **Carpita NC, Kanabus J** (1987) Chemical structure of the cell walls of dwarf maize and changes mediated by gibberellin. Plant Physiol **88:** 671–678
11. **Carpita NC, Shea EM** Linkage structure of carbohydrates by gas chromatography-mass spectrometry (GC-MS) of partially methylated alditol acetates. *In* C Biermann, G McGinnis, eds, Analysis of Carbohydrates by GLC and MS, CRC Press, Boca Raton, FL, pp 156–215
12. **Cleland RE** (1967) Extensibility of isolated cell walls: measurement and changes during cell elongation. Planta **74:** 197–209
13. **Darvill AG, Smith CJ, Hall MA** (1978) Cell wall structure and elongation growth in *Zea mays* coleoptile tissue. New Phytol **80:** 503–516
14. **Dayanandan P, Hebard FV, Kaufman PB** (1976) Cell elongation in the grass pulvinus in response to geotropic stimulation and auxin application. Planta **131:** 245–252
15. **Dische Z** (1947) A new specific color reaction of hexuronic acids. J Biol Chem **167:** 189–198
16. **Dubois M, Gilles DA, Hamilton JK, Rebers PA, Smith F** (1956) Colorimetric method for determination of sugars and related substances. Anal Chem **28:** 350–356
17. **Galambos JT** (1967) The reaction of carbazole with carbohydrates. 1. Effect of borate and sulfamate on the carbazole color of sugars. Anal Biochem **19:** 119–132
18. **Gibeaut DM, Carpita NC** (1990) Improvement of separation of membranes for in vitro synthesis of plant cell wall polysaccharides. Protoplasma (in press)
19. **Hatfield RD, Nevins DJ** (1987) Hydrolytic activity and substrate specificity of an endoglucanase from *Zea mays* seedling cell walls. Plant Physiol **83:** 203–207
20. **Huber DJ, Nevins DJ** (1981) Partial purification of endo- and exo-β-D-glucanase enzymes from *Zea mays* L. seedlings and their involvement in cell wall autohydrolysis. Planta **151:** 206–214
21. **Kato Y, Nevins DJ** (1986) Fine structure of (1→3), (1→4)-β-D-glucan from *Zea* shoot cell walls. Carbohydr Res **147:** 69–85
22. **Kaufman PB, Brock TG, Song I, Rho YB, Ghosheh NS** (1987) How cereal grass shoots perceive and respond to gravity. Am J Bot **74:** 1446–1457
23. **Kvernheim AL** (1987) Methylation analysis of polysaccharides with butyllithium in dimethyl sulfoxide. Acta Chem Scand Ser B **41:** 150–152
24. **Loescher W, Nevins DJ** (1972) Auxin-induced changes in *Avena* coleoptile cell wall composition. Plant Physiol **50:** 556–563
25. **Moscatelli EA, Ham EA, Rickes EL** (1961) Enzymic properties of a β-glucanase from *Bacillus subtilis*. J Biol Chem **236:** 2858–2862
26. **Nelson N** (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem **153:** 375–380
27. **Somogyi M** (1952) Notes on sugar determination. J Biol Chem **195:** 19–23
28. **Song I, Lu C, Brock TG, Kaufman PB** (1988) Do starch statoliths act as the gravisensors in cereal grass pulvini? Plant Physiol **86:** 1155–1162
29. **Woodward JR, Fincher GB, Stone BA** (1983) Water soluble (1→3), (1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. II Fine structure. Carbohydr Polym **3:** 207–225