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CELL WALL β-D-GLUCANS OF FIVE GRASS SPECIES

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Key Word Index—*Zea mays*; *Hordeum vulgare*; *Triticum vulgare*; *Secale cereale*; *Sorghum bicolor*; Gramineae; coleoptile; β-D-glucans.

Abstract—Structural features of noncellulosic β-D-glucans of *Zea mays, Hordeum vulgare, Triticum vulgare, Secale cereale,* and *Sorghum bicolor* were compared. Treatment of cell walls derived from these species with specific *Bacillus subtilis* or *Rhizopus* glucanases yields virtually identical profiles upon Bio-Gel P-2 fractionation of the liberated oligosaccharides. The two predominant reaction products, a trisaccharide and tetrasaccharide, were identified as 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose respectively by virtue of the specificity of these enzymes and by paper chromatography and electrophoresis. The similarity of the reaction product profiles indicates a rather regular repeating sequence in all β-D-glucans examined. The ratios of 3-*O*-β-cellobiosyl-D-glucose to 3-*O*-β-cellotriosyl-D-glucose indicates that 30.4–30.9% of the β-glucosyl linkages in the intact molecule are 1 → 3. The yields of wall glucan as estimated from the quantity of oligosaccharides released, range from 41 μg/mg wall in *Hordeum* to 97 μg/mg wall from *Sorghum*.

INTRODUCTION

Noncellulosic β-D-glucans have been identified as cell wall components of *Zea* [1, 2], *Hordeum* [3, 4], *Sorghum* [5], *Triticum* [6], *Panicum* [7], *Arundinaria* [8], *Secale* [9], *Lolium* [4, 10], and *Avena* [11–14]. These polysaccharides are dissociated from the cell wall matrix of non-endospermic tissues by alkali and have been characterized as linear homopolymers consisting of both β-(1→3) and β-(1→4) glucosidic linkages. The mean ratio of (1→3) to (1→4) linkages has been estimated at 3:7 [15]. Techniques employed in structural characterization of the isolated polymers have included periodate oxidation as in the Smith degradation [8, 12], permethylation followed by acid hydrolysis [8, 12] and partial hydrolysis by either acid [8, 12] or enzymes [2, 6, 8, 11]. Nonetheless, certain structural parameters remain unresolved and a comparison of glucans derived from different species has not been presented. The results reported in this paper indicate that the cell wall β-D-glucan may be liberated as oligosaccharides by specific glucanases. A comparison of these degradation products reveals only minor variations in the linkage sequence of primary cell wall β-D-glucans from the growing tissue of grasses.

RESULTS AND DISCUSSION

Cell walls were prepared from coleoptiles of *Zea*, *Hordeum*, *Triticum*, and *Secale* and from the mesocotyls of *Sorghum* using nonaqueous solvents according to a procedure described by Kivilaan *et al.* [2]. Wall-bound glucosidases were inactivated by heating the wall with 2 ml of water at 100° for 5 min [13]. The water was withdrawn and α-amylase was added to degrade any residual starch which may have adhered to the sample during preparation. After a 20 hr incubation the supernatant fluid including the products of amylase action was removed and the walls were washed with water. Fifteen to twenty mg of wall were incubated for 20 hr with either *Bacillus subtilis*-β-D-glucanase [16, 17] or *Rhizopus* endo-(1→3)-β-D-glucanase [18, 19]. The supernatant fluid was removed by filtration employing glass mat filters and the residue rinsed with water. The combined supernatant and rinse solutions were concentrated to *ca* 1 ml and fractionated on Bio-Gel P-2.

The profiles for all species are similar and reveal that two oligosaccharide fractions are derived from the β-D-glucan in response to treatment with either enzyme. The action pattern of the *B. subtilis* enzyme is restricted to the hydrolysis of the glucosyl linkage at position 1 of the 3 substituted constituent in a β-linkage sequence... Glc(1→4)-Glc-(1→3)-Glc-(1→4)-Glc... [15, 20]. This enzyme is capable of degrading *Avena* and *Hordeum* caryopsis glucans, *Avena* coleoptile cell wall glucan [13, 14] and lichenan but does not hydrolyse laminarin, cellulose or *p*-nitrophenyl-β-D-glucoside [16]. The *Rhizopus* glucanase catalyses the hydrolysis of the glucosyl linkage at position 1 of the 3 substituted constituents of a β-linkage sequence... Glc-(1→3)-Glc-(1→3)-Glc... or Glc-(1→3)-Glc-(1→4)-Glc... [15, 18, 20]. This endo-(1→3)-β-D-glucanase degrades all of the above substrates which are hydrolysed by the *B. subtilis* glucanase but in addition it also acts on laminarin. The predominant products liberated from laminarin upon extended treatment with the enzyme are disaccharides and trisaccharides. The *Rhizopus* enzyme does not hydrolyse cellulose or *p*-nitrophenyl-β-D-glucoside.

The profiles for the five species are virtually identical to those obtained upon treatment of *Avena* coleoptile cell walls with the same two enzymes [14]. The trisaccharide and tetrasaccharide of *Avena* have been identified as 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose respectively. In all cases the similarity of the profiles derived from treatment of walls with two enzymes and the absence of any significant disaccharide in the profile

Table 1. Comparison of β-D-glucans derived from cell walls of five grass species

Species; tissue	Oligosaccharide yield (μg/mg wall)	Molar ratio Tri/Tetra	% 1,3 linkages*
Hordeum coleoptile	41	2.8	30.6
Secale coleoptile	57	3.2	30.9
Triticum coleoptile	65	3.3	30.9
Zea coleoptile	86	3.0	30.8
Sorghum mesocotyl	97	2.5	30.4

* Calculated based on the ratio of tri- and tetrasaccharide assuming that the subunits are linked by a β-1,4 glucosidic bond.

derived upon *Rhizopus* glucanase treatment suggest that regions of the molecule consisting of repeating (1→3)-β-D-glucosidic linkages represent at most only a small proportion of the total glucan complement.

The tentative identification of the liberated trisaccharide and tetrasaccharide was supported by both PC and borate electrophoresis. The single trisaccharide cochromatographed and electrophoretically comigrated with authentic 3-*O*-β-cellobiosyl-D-glucose. A similar analysis served to identify the tetrasaccharide as 3-*O*-β-cellotriosyl-D-glucose.

The apparent similarity in the β-D-glucans from various species indicates a highly conserved glucosyl sequence in the plant family Gramineae. We calculate that in all species examined there are slightly more than 30% (1→3)-β-D-linkages (Table 1). The total yields of β-D-glucan upon treatment of a particular sample with both enzymes are similar; however, quantitative differences exist in walls derived from various species. Current studies on *Avena* β-D-glucan indicate that the yield may also be a function of tissue age.

In order to assess the efficiency of enzyme-mediated β-D-glucan extraction, walls previously treated with the enzyme were incubated for a second time with the β-D-glucanase. No additional release of oligosaccharides was noted.

Physiological interest in the β-D-glucan is based on the observation that a rapid decline in the quantity of this wall component is associated with indole-3-acetic acid-induced growth of coleoptile tissue [13]. Furthermore, changes in the (1 → 3):(1 → 4) linkage ratio have been noted during tissue maturation [12]. The application of these specific enzymes should serve to reveal additional details concerning the nature of the β-D-glucan in cell walls and should assist in the interpretation of any potential physiological function.

EXPERIMENTAL

Preparation of cell walls. Imbibed seeds were placed on 5 mm thick glass plates (30 × 100 mm) which had been covered with absorbant tissue and positioned in a glass tray. H_2O was added to a depth of 2–3 mm, the trays were sealed with plastic film ('Saran Wrap') and illuminated with dim red light at 26° for 48 hr. After 48 hr the seedlings were maintained in complete darkness for the duration of the expt. When the plumule had grown to a length of 30–35 mm the coleoptile was excised and the apex (4 mm portion) and enclosed leaf discarded. The entire mesocotyl of *Sorghum* seedlings (excluding nodal tissue) was excised. The intact tissues were frozen and stored at −20°.

Cell walls were prepared by the method of ref [2]. Frozen tissue segments were homogenized with glass beads (200 μm dia) in cold glycerol by use of a Sorvall Omni-Mixer operated at maximum speed. During homogenization the temp was maintained below 5°. The cell walls and glass beads were collected by filtering the homogenate through Miracloth. The retained material was resuspended in glycerol and filtered. The walls and beads were then added to cold EtOH (−20°) and suspended cell walls decanted and collected on a sintered glass filter. The walls were then rinsed × 3 with cold EtOH followed by 3 rinses with cold Me_2CO (−20°). The collected insoluble material was dried *in vacuo* in the presence of P_2O_5.

Wall analysis. Dried wall (30–40 mg) was suspended in 2 ml of H_2O and heated for 5 min at 100° to inactivate the glucosidases which remain associated with walls during extraction. H_2O was removed and 150 units of porcine pancreatic α-amylase (Sigma Type 1A) added in 1 ml of 25 mM K-Pi buffer (pH 7). The walls were incubated in the presence of α-amylase for 20 hr at 30°. A drop of toluene was added to suppress microbial growth. After amylase treatment cell walls were collected on a glass mat filter (Whatman GF/C) and dried *in vacuo*.

Glucanase degradation of walls. The β-D-glucanase was prepared by chromatographic fractionation (Bio-Gel, P-200) of a commercial *Bacillus subtilis* α-amylase preparation (Sigma Type IIIA). Details of the isolation procedure and characterization of the enzyme have been reported elsewhere [16]. The endo-β-1,3-glucanase was purified from a crude *Rhizopus* glucoamylase preparation (Sigma Chemical Co.) employing a procedure similar to that reported in ref. [18]. The reaction mixtures included 7 μg of *Bacillus* β-D-glucanase in 1 ml Na-Pi buffer (25 mM, pH 6.5) or 15 μg of *Rhizopus* endo-β-1,3-glucanase in McIlvaine buffer (1 ml, pH 4.6) [21]. Cell walls were incubated in the presence of the glucanases for 20 hr at 30°; a drop of toluene was included. The reaction was terminated by heating at 100°. Soluble oligosaccharides were recovered by filtration on 'Whatman' glass mats which retained the insoluble wall. Fractionation of the liberated oligosaccharides was achieved on a 1.5 × 90 cm Bio-Gel P-2 column (200–400 mesh) at 25°. The column eluate was continuously monitored with a differential refractometer. Fractions (1 ml) were collected and the carbohydrate content determined by use of the PhOH–H_2SO_4 method [22].

Chromatography and electrophoresis Trisaccharide and tetrasaccharide components of the Bio-Gel P-2 profile were concentrated to *ca* 0.5 ml. The sugars in these individual fractions were examined by descending PC on Whatman No. 1 paper in the solvent system Py–EtOAc–H_2O (2:8:1) at 26° for 90–135 hr. Alkaline $AgNO_3$ was used to detect individual components [23]. Identification was made by comparison with the following standards which are given with their respective $R_{laminaribiose}$: 3-*O*-β-cellotriosyl-D-glucose, 0.04; cellotriose, 0.16; 3-*O*-β-cellobiosyl-D-glucose, 0.27; gentiobiose, 0.43; laminaritriose 0.44; and cellobiose, 0.66.

Paper electrophoresis was conducted using Whatman No. 1 with 0.2 M Na-borate buffer at pH 10 at 40 V/cm for 90 min. Aniline-phosphate reagent was used to detect individual components [24]. Identification was made by comparison with the following standards and their respective $M_{glucose}$; cellobiose, 0.28; 3-*O*-β-cellotriosyl-D-glucose 0.52; 3-*O*-β-cellobiosyl-D-glucose, 0.58; laminaribiose, 0.70; and gentiobiose, 0.75. 2,3,4,6-Tetra-*O*-methyl-D-glucose was used as an origin reference.

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REFERENCES

1. Buchala, A. J. and Meier, H. (1973) *Carbohyd. Res.* **26**, 421.

2. Kivilaan, A., Bandurski, R. S. and Schulze, A. (1971) *Plant Physiol.* **48**, 389.
3. Buchala, A. J. and Wilkie, K. C. B. (1974) *Phytochemistry* **13**, 1347.
4. Smith, M. M. and Stone, B. A. (1973) *Phytochemistry* **12**, 1361.
5. Woolard, G., Rathbone, P. and Novellie, L. (1976) *Carbohyd. Res.* **51**, 249.
6. Buchala, A. J. and Wilkie, K. C. B. (1973) *Phytochemistry* **12**, 499.
7. Buchala, A. J. (1974) *Phytochemistry* **13**, 2185.
8. Wilkie, K. C. B. and Woo, S. (1976) *Carbohyd. Res.* **49**, 399.
9. Buchala, A. J. and Wilkie, K. C. B. (1970) *Naturwissenschaften* **57**, 496.
10. Smith, M. M. and Stone, B. A. (1973) *Biochim. Biophys. Acta* **313**, 72.
11. Buchala, A. J. and Wilkie, K. C. B. (1971) *Phytochemistry* **10**, 2287.
12. Fraser, C. G. and Wilkie, K. C. B. (1971) *Phytochemistry* **10**, 199.
13. Nevins, D. J., Huber, D. J., Yamamoto, R. and Loescher, W. (1977) *Plant Physiol.* **60**, 716.
14. Yamamoto, R. and Nevins, D. (1978) *Carbohyd. Res.* **53**, in press.
15. Stone, B. A. (1976) *Chem. in N.Z.*, 93.
16. Huber, D. J. and Nevins, D. J. (1977) *Plant Physiol.* **60**, 300.
17. Parrish, F. W., Perlin, A. S. and Reese, E. T. (1960) *Can. J. Chem.* **38**, 2094.
18. Marshall, J. J. (1974) *Carbohyd. Res.* **34**, 289.
19. Reese, E. T. and Mandels, M. (1959) *Can. J. Microbiol.* **5**, 173.
20. Anderson, M. A. and Stone, B. A. (1975) *FEBS Letters* **55**, 202.
21. McIlvaine, T. C. (1921) *J. Biol. Chem.* **48**, 183.
22. Hodge, J. E. and Hofreiter, B. T. (1962) in *Methods in Carbohydrate Chemistry* (Whistler, R. K., ed.), Vol. 1, p. 388.
23. Trevelyan, W. E., Procter, D. P. and Harrison, J. S. (1950) *Nature* **166**, 444.
24. Bryson, J. L. and Mitchell, T. J. (1951) *Nature* **167**, 864.