Purification of an Endo-β-1,4 Galactanase Produced by Sclerotinia sclerotiorum: Effects on Isolated Plant Cell Walls and Potato Tissue

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ABSTRACT

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Sclerotinia sclerotiorum produces an endo-β-1,4 galactanase when grown in a liquid mineral salts medium supplemented with 2.0 g of Difco yeast extract and 2.0 g or 6.0 g D-galactose per liter. This enzyme was purified by CM Sephadex ion exchange chromatography and by Sephadex G-50 and G-75 gel filtration. The purified enzyme has an isoelectric point of 8.3, a pH optimum of approximately 4.5, and a molecular weight of 22,000 to 24,000 daltons. This enzyme, free of endopolygalacturonase or pectic lyase activity, readily solubilized substantial amounts of carbohydrate including a major portion of the galactan component from isolated sycamore and potato cell walls. However, endo-β-1,4 galactanase, at a concentration of 0.3 units/ml, did not cause maceration of potato tuber tissue disks during a 20-hr exposure at pH 5.0, even though some galactose was released from the tissue.

Many phytopathogenic fungi and bacteria produce enzymes capable of hydrolyzing the polymeric carbohydrate constituents of higher plant cell walls. These polysaccharidases are normally inducible, extracellular, and often highly stable. A number of different cell-wall-degrading enzymes can be readily detected in plant tissues infected with facultative pathogens (4).

When grown on plant cell wall material, plant pathogens commonly produce a variety of polysaccharidases (6, 13, 19, 29). The principal kinds of enzymes produced are believed to correspond to the major types of glycosidic linkages present in the polysaccharides of the plant cell wall material used as the carbon source. There is evidence that the monosaccharide or oligosaccharide fragments formed by enzymatic hydrolysis of a particular polysaccharide can serve as effective inducers of specific hydrolases, or of a set of related enzymes (11). Recent studies in which isolated plant cell walls have been used as carbon sources for plant pathogens, suggest that the cell-wall-degrading enzymes may be produced in a temporal sequence, with polygalacturonases being first and cellulases being last in the sequence (13, 19, 29).

Highly purified preparations of endopolygalacturonases and endoglucanases (cellulase) have proven to be useful tools in the elucidation of higher plant cell wall structure (8, 21, 33). Purified endo-pectic enzymes readily degrade isolated plant cell walls. The action of these enzymes on isolated cell walls appears to render the nonuronide polymers more accessible or susceptible to hydrolysis by other enzymes (20, 33). The pectic enzymes are known to be responsible for tissue maceration (4). During the process of tissue maceration, cell walls become unable to support the plant protoplast under osmotic stress (2, 32). This dual effect of pectic enzymes on plant tissues gives these enzymes an important role in many types of tissue decay. The depletion or alteration of cell wall polysaccharides following pathogenic infection has been demonstrated in a number of suscept-pathogen systems (3, 16, 25). Aside from the pectic enzymes and cellulase, the specific contributions of individual cell-wall-degrading enzymes to plant cell wall or tissue breakdown are poorly understood. The current view of primary cell wall structure holds that an arabinogalactan containing β-1,4 linked galactose is a key constitutent linking the rhamnogalacturonan fraction to the hemicellulosic fraction in primary cell walls (21). The availability of a purified endo-β-1,4 galactanase could aid structural studies of plant cell walls as well as permit studies on the role of galactanases in cell-wall degradation.

This is a report of the partial purification and properties of an endo-β-1,4 galactanase produced by *Sclerotinia sclerotiorum*, and the effects of this enzyme on isolated plant cell walls and potato tissue. A preliminary report of this work has been published (7).

MATERIALS AND METHODS

Substrates and chemicals.—Lupine galactan was isolated from the seeds of *Lupinus albus* by the method of Jones and Tanaka (18). Soy galactan was prepared from soybean flour as described by Morita (26). Pectin and sodium polypectate were obtained from Sunkist Growers, Inc., Sherman Oaks, CA 91403; and carboxymethyl cellulose (type 7MP) from Hercules

Powder Corp., Wilmington, DE 19899. Crude xylan from larch was purchased from Sigma Chemical Co., St. Louis, MO 63178, and purified by precipitation of the contaminating glucomannan with barium hydroxide as described by Timell (34).

Meta-hydroxydiphenyl was purchased from K & K Laboratories, Inc., Plainview, NY 11803. Sugars, oligosaccharides, and *p*-nitrophenyl-β-D-galactoside were purchased from Sigma Chemical Co. Para-nitrophenyl-α-L-arabinofuranoside was the gift of Peter Albersheim. All other chemicals were reagent grade.

Cell walls from log-phase suspension cultured sycamore (*Acer pseudoplatanus*) cells were prepared as described by Talmadge et al. (33). Cell walls also were prepared from medular tissue of Katahdin potato tubers. The tissue was diced and washed with ice-cold 500 mM potassium phosphate buffer, pH 7.0, containing 1% ascorbic acid. The diced tissue then was ground with liquid nitrogen in a mortar to a fine powder which contained no intact cells. The powder was washed extensively with the above buffer and distilled water on a sintered glass filter. Starch was removed by treatment with 1 mg α-amylase (type IIA, Sigma Chemical Co.) per 100 gm tuber tissue in 50 mM potassium buffer, pH 7.0. Sodium azide (0.02%) was added during amylase treatment to prevent microbial growth. After 12 hr of incubation with stirring at room temperature in the presence of amylase, the wall suspension was sonicated for 10 min in an ultrasonic bath and then filtered through a coarse sintered glass filter to remove small, undegraded starch particles. Small particles of starch also were removed by decantation after allowing the wall particles to settle. The potato cell wall material then was washed with 95% ethanol and water prior to the next amylase treatment. Three-to-five such treatments with amylase generally were required to remove all of the starch, as indicated by lack of staining with iodine. The starch-free cell walls were washed several times with 1:1 chloroform-methanol and with acetone, then air dried.

Preparation of culture filtrates.—*Sclerotinia sclerotiorum* (Lib.) de Bary (isolate 214) [*Whetzelinia sclerotiorum* (Lib.) Korf & Dumont] used in this study was isolated from a diseased carrot by O. C. Yoder and identified by R. P. Korf and Linda Kohn. Although this isolate grew well on potato-dextrose agar (PDA), repeated transfers on PDA resulted in the loss of its ability to produce large quantities of galactanase in liquid media. Therefore, stock cultures on PDA were stored under distilled water at 4 C and transfers from these cultures to fresh PDA were made as needed.

For galactanase production, *S. sclerotiorum* was grown in a liquid medium containing per liter, 181 mg $MgSO_4$, 149 mg KCl, 1.0 g NH_4NO_3, 650 mg KH_2PO_4, 3.5 mg $ZnSO_4 \cdot H_2O$, 6.9 mg $MnSO_4 \cdot H_2O$, 3.1 mg $CuSO_4 \cdot 5H_2O$, 2.0 g Difco yeast extract, and 2.0 or 6.0 g D-galactose. One liter of medium was autoclaved for 20 min at 1 atmosphere gauge pressure (15 psi) in 2.8-liter Fernbach flasks. Cultures were started with water suspensions of mycelial fragments scraped from the surface of PDA plates on which the fungus had grown for 7 days at 25 C. The liquid cultures were incubated at 25 C without shaking for 14 to 20 days. The fungal mats were removed from the medium by passing the filtrates through several layers of cheesecloth, followed by filtering through two layers of glass fiber filter paper. This filtrate was dialyzed overnight against distilled water or 50 mM sodium acetate (pH 5.0) at 4 C and designated crude culture filtrate.

Enzyme assays.—Galactanase, xylanase, carboxymethylcellulase (endo-glucanase), and polygalacturonase activities were assayed at 30 C in reaction mixtures containing 0.9 ml of a 0.1% polysaccharide substrate in 50 mM sodium acetate (pH 4.5) plus enzyme and water to make a final volume of 1.0 ml. Enzyme activities were determined by release of reducing groups from specific substrates as determined by the Somogyi-Nelson procedure (30); the standard curve was prepared with galactose. Beta-galactosidase and α-L-arabinofuranosidase activities were assayed in reaction mixtures containing 1.9 ml of 0.05% *p*-nitrophenyl-β-D-galactoside or 0.02% *p*-nitrophenyla-α-L-arabinofuranoside in 50 mM sodium acetate, pH 4.5, plus enzyme and water to give a final volume of 2.0 ml. Enzyme activity was terminated by adding 1.0 ml of 1 M NH_4OH and measured by determining the increase in absorbance of the reaction mixture at 400 nm; *p*-nitrophenol was used as the standard. For all enzyme assays, one unit of activity was defined as that amount of enzyme which released 1 μmole of product (reducing sugar or *p*-nitrophenol) per min under the assay conditions described.

Protein was determined by the procedure of Lowry et al. (24); bovine serum albumin was used as the standard.

Isoelectric focusing (pH 3-10) of enzymes from the crude culture filtrates and the determination of tissue maceration and cell death in potato tuber tissue were performed as described by Mount et al. (27). Disc gel electrophoresis of the endo-β-1,4 galactanase at various stages of purification was performed according to the methods described by Gabriel (15), using the pH 3.8 buffer system and 7.5% acrylamide gels.

Fractionation of the reaction products obtained from endo-β-1,4 galactanase hydrolysis of soy and lupine galactans was carried out by descending paper chromatography on Whatman No. 1 paper irrigated with butanol:acetic acid:water (2:1:1, v/v). Silver nitrate reagent (31) was used to locate reaction products on chromatograms. Reaction products were also fractionated by gel filtration in a 1.5 × 95 cm column of BioGel P-2 (BioRad Laboratories, Richmond, CA 94804) maintained at 52 C. The column was equilibrated and eluted with water. Galactose, melibiose, raffinose, and stachyose were used as reference standards in these fractionations.

Determination of sugars and polysaccharide composition.—Uronic acids were estimated by the *m*-hydroxydiphenyl procedure of Blumenkrantz and Asboe-Hansen (9); galacturonic acid was used as the standard. Hexoses were determined by the anthrone procedure (12), and galactose served as the standard.

The neutral sugar compositions and methylation analysis of polysaccharides were determined using the procedures described by Lindberg (23), except for the hydrolytic procedure. Hydrolysis of the polysaccharides (1- to 5-mg samples) was effected with 1.0 ml of 2 N trifluoroacetic acid in sealed glass tubes held at 120 C for

1-3 hr; samples were evaporated to dryness with a stream of filtered air.

Gas chromatography was carried out with a Perkin-Elmer Model 900 instrument equipped with dual flame ionization detectors and a linear temperature program. Peak areas were measured by triangulation. Glass columns (1.8 m long, 2 mm I.D.) packed with 0.2% ethylene glycol adipate, 0.2% ethylene glycol succinate, and 0.2% GE XF 1150 liquid phase on 149-131 μm (100/120-mesh) Gas Chrome Q (column a) were used for gas chromatography of the alditol acetate sugar derivatives. This column packing, and 3% ECNSS-M on Gas Chrome Q (column b), were used for gas chromatographic separation of the nonmethylated or partially methylated alditol acetate derivatives, as described by Talmadge et al. (33). Column packing materials were obtained from Applied Science, Inc., State College, PA 16801.

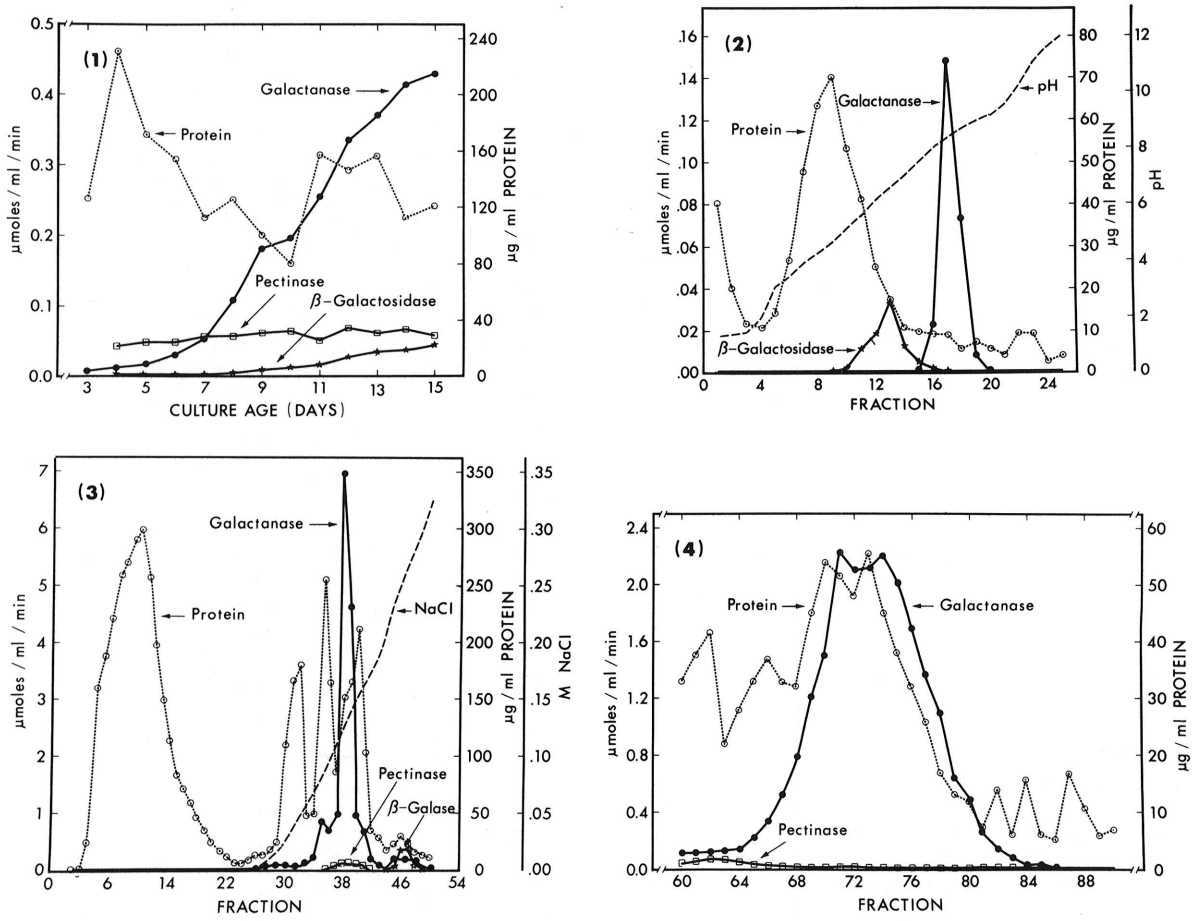

Fig. 1-4. **1)** Production of galactanase by *Sclerotinia sclerotiorum*. One-liter still cultures of the *S. sclerotiorum* isolate were grown at 25 C on medium containing 0.2% galactose as the sole carbon source. Samples were withdrawn daily, filtered free of fungal mycelia, dialyzed to remove monosaccharides, and assayed for total protein and galactanase, pectinase and β-galactosidase activities. **2)** Isoelectric focusing of galactanase produced by *S. sclerotiorum*. Crude culture filtrate, from cultures grown on medium containing 0.2% galactose as the sole carbon source, was concentrated by ultrafiltration and subjected to isoelectric focusing in an LKB apparatus for 3 days at 4 C. Five-ml fractions were collected, their pH determined, and each was dialyzed against water at 4 C. Aliquots of the dialyzed fractions were assayed for total protein and for galactanase, pectinase and β-galactosidase; there was no detectable pectinase. **3)** Fractionation of galactanase from *S. sclerotiorum* on Sephadex CM-50. Crude culture filtrate (1.56 liter) was concentrated by ultrafiltration to 150 ml, dialyzed against 20 mM sodium acetate, pH 5.0, and applied to a 2.5 × 42.5-cm bed of Sephadex CM-50 equilibrated with the same buffer. The column was eluted with 200 ml of buffer and then with a linear gradient (400 ml buffer mixed with 400 ml buffer containing 400 mM sodium chloride). Twenty-ml fractions were collected and analyzed for sodium chloride concentration (by conductance), total protein, and galactanase, pectinase and β-galactosidase (β-galase). When similar colums were assayed for α-arabinosidase, this activity appeared as a single peak eluting in a position corresponding to fractions 34-36. **4)** Gel filtration of *Sclerotinia* galactanase on Sephadex G-50. The fractions containing substantial galactanase activity from the CM-Sephadex ion-exchange column (Fig. 3) were pooled, concentrated 20-fold by ultrafiltration, and fractionated on a 1.9 × 93.6-cm column of Sephadex G-50 (fine) equilibrated with 50 mM sodium acetate, pH 4.5, containing 100 mM sodium chloride. Fractions of 1.5 ml were collected at the flow rate recommended by Pharmacia ("Gel Filtration in Theory and Practice", Pharmacia Fine Chemicals). Fractions were assayed for total protein, galactanase and pectinase.

RESULTS

Production of galactanase.—Culture medium containing 0.2 or 0.6% galactose as the carbon source was seeded with *S. sclerotiorum* and incubated for 14 to 21 days at 25 C. Aliquots (5 ml each) of culture fluid were removed daily, dialyzed against 4 liters of 50 mM sodium acetate, pH 4.5, for 2-3 hr to remove galactose, centrifuged to remove suspended matter, and then assayed in duplicate for total protein and for galactanase, β-galactosidase, and polygalacturonase (Fig. 1). Duplicate cultures yielded similar results for galactanase production, but both the highest galactanase activity reached and the time required to reach maximum activity exhibited some variation between sets of cultures. Cultures grown in media containing 0.2% galactose had a shorter lag time before significant galactanase activity appeared than those grown in media containing 0.6% galactose, and they reached maximal galactanase activity more quickly. This suggests that the galactanase is not produced until the concentration of galactose in the culture medium has been reduced to a low level.

Crude culture filtrates (1,900 ml) were dialyzed twice (16 hr each time) against 8 liters of 20 mM sodium acetate, pH 5.0. The dialyzed culture filtrates were concentrated either by ultrafiltration using a UM-10 membrane or by batchwise addition of 50 ml of CM Sephadex C-50 (equilibrated with 20 mM sodium acetate, pH 5.0) per liter of dialyzed culture filtrate. The CM Sephadex was stirred with the culture filtrate for 1 hr at 4 C, filtered through Whatman No. 1 filter paper on a Büchner funnel, poured into a 2.6 cm I.D. column and washed with 20 mM sodium acetate buffer, pH 5.0. The column then was eluted with buffer containing 200 mM sodium chloride, and 10-ml fractions were collected. Those fractions containing significant galactanase activity were pooled and dialyzed overnight against 4 liters of 20 mM sodium acetate, pH 5.0.

Protein in samples of crude culture filtrate, concentrated by ultrafiltration, were fractionated by isoelectric focusing (Fig. 2). The galactanase activity, assayed using lupine galactan as the substrate, appeared in a single, well defined peak corresponding to a protein with an isoelectric point of approximately 8.3.

Enzyme purification.—Concentrated culture filtrate in 20 mM sodium acetate, pH 5.0, was applied to a 2.5×42.5 cm column of CM Sephadex C-50 pre-equilibrated with the same buffer. The column was eluted with a linear salt gradient using 400 ml each of buffer and 400 mM NaCl in buffer; 20-ml fractions were collected. Samples of each fraction were assayed for protein, galactanase, endopolygalacturonase, and β-galactosidase (Fig. 3). Fractions 38 and 39, which contained the bulk of the galactanase activity, were pooled and reassayed. This step gave a 9-fold purification of the galactanase with 70% recovery of the initial activity.

The pooled fractions were concentrated by ultrafiltration, and this concentrated galactanase prepration was then subjected to gel filtration in a column (1.9 × 93.6 cm) of Sephadex G-50 (fine). This procedure usually separated the galactanase from the remaining traces of endopolygalacturonase activity (Fig. 4). Fractions 68-79 were pooled and reassayed. Recovery of galactanase activity was over 80% of that applied to the column, with a 1.5- to 2-fold increase in specific activity.

The molecular weight of the galactanase was estimated by its elution volume from a column of Sephadex G-75 in which bovine serum albumin (m.w. 67,000), ovalbumin (m.w. 43,000), peroxidase (m.w. 40,000), sytochrome c (m.w. 12,300), and myoglobin (m.w. 17,500) were used as reference standards. The galactanase eluted at a volume corresponding to a globular protein with a molecular weight of approximately 22,000 to 24,000 daltons. In some cases the Sephadex G-75 column was used as an additional purification step. Pooled fractions from the Sephadex G-50 column were concentrated and layered onto a 1.9×89.5 cm column of Sephadex G-75 and eluted with 0.05 M sodium acetate pH 5.0 + 0.1 M NaCl. Fractions free of endopolygalacturonase activity were pooled and designated purified galactanase.

Disc gel electrophoresis of the galactanase after CM Sephadex C-50 and Sephadex G-75 chromatography revealed that such galactanase preparations contained three or four protein impurities. Although the galactanase was not homogeneous, the purified enzyme contained no detectable endopolygalactanase, xylanase, carboxymethylcellulase, α-arabinofuranosidase, or β-galactosidase activity.

Enzyme characterization.—The purified galactanase gave a linear assay response with the lupine galactan to 0.8 μmoles of reducing groups released, which corresponds to approximately 15% of the total glycosidic bonds present in the substrate. The pH optimum was 4.5 to 5.0. The presence of Ca^{++}, Mg^{++}, or EDTA in concentrations up to 1 mM had no appreciable effect on enzyme activity.

The purified galactanase in 50 mM sodium acetate, pH 5.0, (containing 100 mM NaCl) could be stored at 4 C for several weeks without significant loss in activity. Most of the activity was preserved in freeze-dried preparations after several weeks of storage, but very little activity remained in frozen preparations stored at −20 C.

Both soy and lupine galactan preparations were used to examine the pattern of action of the purified galactanase. The sugar compositions of both substrates were determined (Table 1). Methylation analysis of lupine galactan (Bauer et al., *unpublished*) showed that over 60% of the recovered carbohydrate corresponded to 1,4-linked galactose and 10% of the recovered carbohydrate corresponded to a derivative arising from nonreducing, terminal galactose residues. The galactanase hydrolyzed both galactan substrates and released the same type of reaction products from each. Paper chromatograms of reaction products after different periods of hydrolysis revealed that the reaction products included monomeric galactose and a series of oligosaccharides.

Further experiments were carried out in which 20 mg of either the soy or lupine galactan were incubated with the purified galactanase until no further increase in reducing groups were detected. This point was reached with approximately 27% hydrolysis of the soy galactan and approximately 32% hydrolysis of the lupine galactan. Reaction products from such exhaustive hydrolysis were fractionated in BioGel P-2 columns (Fig. 5). The elution profile of reaction products from soy galactan was similar to that shown in Fig. 5 except that two to three times more

material appeared in the peak eluting near the void volume of the column, and there was less product in each of the subsequent peaks. The sugar compositions of carbohydrate material from the five indicated regions in Fig. 5 are given in Table 1. These results, as well as those obtained by paper chromatography of the reaction products, indicate that the purified enzyme is capable of hydrolyzing internal glycosidic linkages between galactosyl residues of the 1,4-linked polymers.

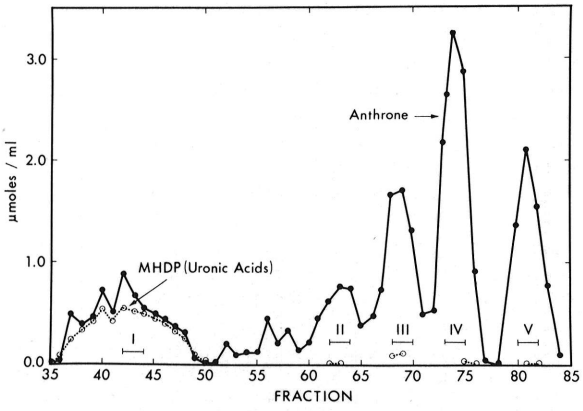

Fig. 5. Fractionation of lupine galactan on BioGel P-2 after hydrolysis with *Sclerotinia* galactanase. Purified *Sclerotinia* galactanase (100 µl) from the Sephadex G-50 column (Fig. 4) was reacted exhaustively (7 hrs) with 20 mg of lupine galactan in 900 µl of 50 mM sodium acetate, pH 5.0, at 23 C. The reaction mixture was then applied to a 1.5×95-cm column of BioGel P-2, equilibrated and eluted with water at 52 C. Fractions of 1.5 ml were collected at a flow rate of 12 ml/hr and assayed by the *m*-hydroxydiphenyl method (MHDP) for uronic acids and the anthrone method for neutral sugars, primarily hexoses. Galacturonic acid and galactose were used as standards for these assays, and the results are shown in galacturonic acid- or galactose-equivalent units. Pooled fractions corresponding to the five regions designated by Roman numerals I-V were taken for analysis of neutral sugar composition. A mixture of galactose, melibiose, raffinose, stachyose and dextran was fractionated on the same column in order to determine the elution volumes of mono-, di-, tri-, and tetrasaccharides and the void volume.

Effects of galactanase on isolated plant cell walls and potato tuber tissue.—Isolated cell walls from potato tuber tissue and suspension cultured sycamore cells were subjected to hydrolysis by the purified galactanase from *S. sclerotiorum*. The reaction mixtures contained 20 mg of wall material suspended in 50 mM sodium acetate, pH 5.0, and five units of enzyme in a total volume of 4.0 ml. Reaction mixtures were incubated at 25 C. A time-course examination of the release of soluble carbohydrate as detected by the anthrone and Somogyi-Nelson methods, revealed that hydrolysis reached a maximum within a period of 3 hr. After 3 hr, 1 mg of galactose-equivalent carbohydrate was released from 20 mg (dry wt) of sycamore cell walls and 6.8 mg of galactose-equivalent carbohydrate was released from the same quantity of potato cell walls. The reducing-group assays indicated that 0.2 mg and 2.0 mg of galactose-equivalent reducing groups were released from 20 mg each of sycamore and potato cell walls, respectively.

Disks (9 mm diam, 0.5 mm thick) of potato tuber medullar tissue were incubated with the purified endo-β-1,4 galactanase for periods up to 24 hr at about 26 C. Reaction mixtures contained 1.0 unit of galactanase per ml in 50 mM sodium acetate, pH 5.0, and 15 tissue disks in a total volume of 3.0 ml. Sodium azide (0.02%) was added to prevent microbial growth. The endopectate lyase (1) used as a control in these studies macerated the potato tuber tissue disks within a period of 0.5 hr when used at a concentration of 0.3 units per ml. In several different experiments the purified galactanase failed to macerate potato tuber tissue disks. The failure of galactanase to cause tissue maceration was not due to inactivation of the enzyme since approximately 70% of the enzyme activity could be demonstrated in the reaction mixtures after 24 hr of incubation.

DISCUSSION

Evidence is provided that the galactanase purified in this study is an *endo* enzyme capable of hydrolyzing internal galactosidic linkages between galactosyl residues of a β-1,4-linked galactan polymer (Table 1 and Fig. 5). This enzyme from *S. sclerotiorum* appears to hydrolyze the substrate in an essentially random fashion, producing a mixture of mono-, di-, and trisaccharides of galactose; it

TABLE 1. Neutral sugar compositions of lupine and soy galactans and of BioGel P-2 fractions of lupine galactan hydrolysate[a]

Sugar	Weight percent						
	BioGel P-2 fraction					Lupine galactan	Soy galactan
	Void peak	Oligosaccharide peaks					
	I	II	III	IV	V		
Rhamnose	5.0	0.0	0.0	0.0	0.0	3.3	4.9
Fucose	0.0	0.0	0.0	0.0	0.0	0.0	2.7
Arabinose	58.9	11.3	7.3	3.8	0.0	9.4	33.5
Xylose	14.3	0.0	0.0	0.0	0.0	4.9	6.6
Galactose	21.8	88.6	92.7	96.2	100.0	82.5	50.2
Glucose	0.0	0.0	0.0	0.0	0.0	0.0	2.0

[a]Lupine galactan was exhaustively hydrolyzed with purified galactanase from *Sclerotinia sclerotiorum* and the reaction products were fractionated by gel filtration on BioGel P-2. Peaks of carbohydrate were pooled (Roman numerals I-V) and analyzed by gas chromatography. The neutral sugar compositions of the carbohydrate in each of the five regions, and of untreated lupine and soy galactans, is given as weight percent of the total carbohydrate detected by gas chromatography.

apparently can hydrolyze galactose tetramers, but not trimers. The pattern of hydrolysis of galactan observed for this endo-β-1,4 galactanase is quite similar to that reported for the action of the endopolygalacturonase of *Colletotrichum lindemuthianum* on polygalacturonic acid (14).

The endo-β-1,4 galactanase of *S. sclerotiorum* is readily purified from culture filtrates of the fungus grown on a medium containing galactose as the carbon source (Fig. 3 and 4). Galactose appears to serve as the inducer for galactanase production, but this sugar may repress enzyme synthesis when present above a certain minimum concentration. This was indicated by the fact that a lag was observed prior to the appearance of galactanase in culture medium containing 0.2 or 0.6% galactose: the lag period for enzyme production was considerably less when the medium contained 0.2% galactose as opposed to the higher concentration. Cooper and Wood (11) have demonstrated that the monomeric sugars from a number of plant cell wall polymers are the inducers of specific polysaccharidases in plant pathogenic fungi.

The 1,4-linked galactosyl residues comprise about 4% of the noncellulosic carbohydrates or about 2.5% of the total dry weight of isolated suspension cultured sycamore cell walls (33). The potato cell wall is a high-galactose material; approximately 45% of the noncellulosic carbohydrate is polymeric galactose (17, 28). Galactose accounts for 23 to 29% of the dry weight of the potato cell wall (28). Recent experiments by Bhuvaneswari and Bauer (*unpublished*) in which isolated potato cell walls were subjected to methylation and compositional analysis revealed that most of the galactose in this structure is 1,4-linked.

The polymeric galactose in isolated sycamore and potato cell walls was made soluble by the purified endo-β-1,4 galactanase of *S. sclerotiorum*. About 5.0% of the isolated sycamore cell walls became soluble within a 3-hr period by the purified enzyme. Since the fraction solubilized exceeds the percentage of these walls known to exist as 1,4-linked galactose (33), it appears that sugars other than galactose were present in the soluble oligomeric carbohydrate. This conclusion is compatible with both our data on the release of reducing groups from sycamore cell walls by the purified galactanase from *S. sclerotiorum* and the reported structure of sycamore cell walls (21). Approximately 35% of the dry wt of the potato cell wall was solublized by the purified endo-β-1,4 galactanase; this amounts to about 56% of the noncellulosic carbohydrates present (28). Based on measurement of the reducing groups in the carbohydrate released by this enzyme, 45-50% of the 1,4 galactosyl linkages in the potato cell wall must have been hydrolyzed.

Evidence has been presented that the action of a "wall modifying enzyme" may be necessary prior to extensive degradation of specific carbohydrate polymers in plant cell walls (20). There is considerable evidence that purified pectic enzymes readily remove major amounts of the uronic acid fraction as well as other carbohydrates from isolated cell wall (2, 14). The view has emerged that the pectic enzymes, particularly the endo enzymes that split the α-1,4 galacturonosyl linkages within the cell wall, function as "wall modifying enzymes" that render other cell wall polymers more accessible or susceptible to enzymatic hydrolysis (4). The work of Bauer et al. (8) showed that the xyloglucan of isolated sycamore cell walls was not readily hydrolyzed by endoglucanase (cellulase) without prior treatment of these walls with an endo-polygalacturonase. In contrast, the current study with endo-β-1,4 galactanase has revealed that the galactan fractions in both isolated sycamore and potato cell walls are accessible to and readily hydrolyzed by this enzyme without the prior action of a pectic enzyme.

The endopectic enzymes represent the only group of polysaccharidases confirmed to cause plant tissue maceration (4). The reported structure of the primary plant cell wall (21) and the high galactose content of the potato cell wall (17, 28) suggest that an endo-β-1,4 galactanase might effect maceration of potato tuber tissue. A preliminary report by Cole and Sturdy (10) indicated that endogalactanases do cause maceration of potato tuber tissue. Our results with a highly purified endo-β-1,4 galactanase fail to support this claim. The failure to effect plant tissue maceration with purified endo-β-1,4 galactanase recently has been confirmed with a purified galactanase from another source (R. C. Codner, *personal communication*). It has been our experience that enzyme preparations which cause plant tissue maceration always contain endopectic enzymes, even if in trace amounts.

A number of plant pathogens are known to produce galactanases in substantial quantities (4, 10, 22, 29, 35). The role, if any, of galactanases in the pathogenicity of these organisms is unknown, but it is apparent that they can function in cell wall alterations during tissue breakdown (5, 16). The availability of a purified endo-β-1,4 galactanase should facilitate studies on the function of this group of enzymes in plant tissue breakdown by pathogens.

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