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Cell Wall Metabolism in Ripening Fruit[1]

III. PURIFICATION OF AN ENDO-β-1,4-XYLANASE THAT DEGRADES A STRUCTURAL POLYSACCHARIDE OF PEAR FRUIT CELL WALLS

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ABSTRACT

A β-1,4-xylanase has been purified from the mixture of carbohydrate-degrading enzymes found in a commercial preparation from cultures of *Trichoderma viride*. Purification from the desalted enzyme mixture is accomplished either by preparative isoelectric focusing or in two-column chromatographic steps. The xylanase has maximal activity at pH 5.0 and a molecular weight of approximately 13,000 daltons. The enzyme loses activity when heated to above 45°C. The xylanase degrades xylans from larch and pear cell walls in an apparently endo-fashion.

Recent investigations of cell wall polysaccharide structure have led to the formulation of models of the interpolymer relationships in the intact wall (18, 19). These models are tentative and must be tested beyond the systems for which they have been proposed. Nevertheless, they provide a structural framework by which more limited observations of development-related wall changes can be assessed (2, 16, 26).

In large part, interpretations of interpolymer relationships have been based on data obtained following digestion of cell wall preparations with purified enzymes which are generally of microbial origin (2, 5, 27). Further development of models may depend largely on the availability of additional enzyme preparations.

Polysaccharides with a β-1,4-xylan backbone have been described for cell walls of monocots and dicots (1, 4, 7, 8, 18). These backbones can be largely unsubstituted or bear variable amounts of arabinosyl and glucuronosyl substituents. Chanda *et al.* (8) have described a glucuronoxylan as a component of the hemicellulose fraction of cell walls from the 'Conference' pear and a similar polymer is apparently present in the 'Bartlett' pear (1). This paper describes the purification of a β-1,4-xylanase from the complex mixture of polysaccharide- and oligosaccharide-degrading enzymes in Cellulysin, a preparation from cultures of the fungus *Trichoderma viride*. Purification can be accomplished in a two-step procedure using conventional column chromatography or in one step using preparative isoelectric focusing which resolves the xylanase into a family of proteins which have pI values considerably more alkaline than the other carbohydrate-degrading enzymes identified in Cellulysin. The use of purified xylanase for partial characterization of Bartlett pear wall xylan is described.

MATERIALS AND METHODS

The preparation used as a source of xylanase was Cellulysin (Calbiochem-Behring). This enzyme mixture is prepared from cultures of the fungus *Trichoderma viride*. Prior to use, the Cellulysin powder was dissolved in water and freed of considerable quantities of salt and low-mol wt carbohydrate by passage through a column of Bio-gel P-10 equilibrated in water. Protein-containing fractions were lyophilized.

Column chromatography media used in this study were SP-Sephadex and Sephadex G-100 (Pharmacia) and Bio-gels P-2 and P-10 (Bio-Rad). Media for isoelectric focusing (Biolyte electrofocusing gel; Biolyte ampholytes 6/8, 7/9, 3/10, and acrylamide) were purchased from Bio-Rad. Calibration standards used in gel filtration were purchased from Pharmacia. Carbowax 20,000 was a product of Union Carbide.

Enzyme Assays. Polysaccharide-degrading activities in the crude enzyme preparation and in chromatography fractions were assayed by measuring the generation of reducing groups according to the method of Nelson (20), as modified by Somogyi (25). The standard reaction mixture contained enzyme and 0.1% (w/v) substrate in 1 ml of 100 mM sodium acetate (pH 5.0). Incubation was at 37°C. Substrates used were larch xylan, CMC[2], laminarin and polygalacturonic acid (Sigma), araban (Koch-Light Laboratories, LTD), and galactan. Prior to use, the xylan was purified according to the technique of McNeil and Albersheim (as described by Taiz and Honigman [26]) to remove contaminating carbohydrates. Araban was freed of contaminating uronosyl residues as described by Ahmed (1). Galactan (β-1,4-linked) was prepared from citrus pectin as described by Labavitch *et al.* (15).

Material, presumably undigested substrate, would precipitate upon development of the reducing group assay when CMC and xylan were used as substrates. Solutions were clarified by centrifugation (5 min, 2,000*g*) in a bench-top centrifuge prior to determining *A* at 520 nm.

Glycosidase activities were assayed by measuring the amount of *p*-nitrophenol released from various *p*-nitrophenyl glycosides following incubation at 37°C. The standard reaction mixture contained enzyme and 0.1% (w/v) substrate in 1 ml of 100 mM acetate (pH 5.0). Incubation was terminated by addition of 2 ml of 1 M NH_4OH containing 2 mM EDTA. Free *p*-nitrophenol was measured by reading *A* at 400 nm. The *p*-nitrophenyl substrates used were α- and β-D-galactoside, α- and β-D-glucoside, and α-D-mannoside (Sigma); α- and β-L-fucoside and α- and β-D-xyloside (Koch-Light Laboratories, LTD); and α-L-arabinoside (prepared according to the technique of Fielding and Hough [10]).

Extended incubations were carried out in the presence of toluene to retard microbial growth.

Preparation and Extraction of Cell Walls. Cell walls were prepared from mature green Bartlett pears and treated with a preparation of *Aspergillus niger* PG that was free of contaminating hydrolases (2). Following PG treatment, the residue was washed in water and lyophilized. Twenty mg of dried residue were sus-

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[2] Abbreviations: CMC, carboxymethylcellulose; PG, polygalacturonase.

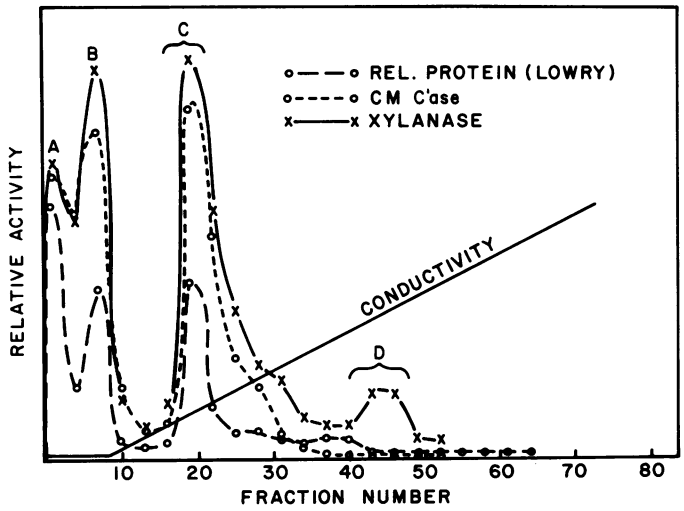

FIG. 1. SP-Sephadex chromatography of desalted Cellulysin. The column (15- × 120-mm gel bed) was equilibrated in 20 mM sodium acetate (pH 4.0). The enzyme sample (120 mg in 20 ml of 20 mM acetate) was applied and the column was eluted with 100 ml of 20 mM acetate and then with a linear gradient produced by mixing 400 ml each of 20 mM acetate and 20 mM acetate containing 500 mM NaCl. Fractions of 10 ml were collected and assayed. Peaks of xylanase activity which void the column and which are eluted at successively higher NaCl concentrations are indicated by A, B, C, and D.

Table I. *K_{av} Values for Various Carbohydrate-Degrading Enzymes in Desalted Cellulysin Chromatographed on Sephadex G-100, as Described for SP-Sephadex-Purified Xylanase*

K_{av} values for standards and their mol wt are provided for comparison. $K_{av} = \frac{V_e - V_o}{V_t - V_o}$, where V_e = elution volume of test protein, V_t = column bed volume, and V_o = column void volume. Minor activities identified in the desalted preparation, but not followed following gel filtration, are α-xylosidase, α-mannosidase, β-galactosidase, and 'azocaseinase.'

Protein	K_{av}	Mol Wt[a]
Standards		
Ribonuclease	0.652	13,700
Chymotrypsinogen	0.526	25,000
Ovalbumin	0.320	45,000
BSA	0.208	67,000
Enzymes		
β-Xylosidase	0.211	66,000
β-Glucosidase	0.256	56,200
α-Galactosidase	0.288	47,900
β-1,3-Glucanase	0.288	47,900
CMC'ase	0.396	34,700
β-1,4-Xylanase I	0.396	34,700
Polygalacturonase	0.411	33,100
α-Arabinosidase	0.456	28,200
β-1,4-Xylanase II	0.674	13,200

[a] Mol wt for enzymes approximated from plot of log mol wt *versus* K_{av} for standards.

pended in 5 ml of 4.4 M NaOH containing 1 mg sodium borohydride per ml in a screw-capped tube. The sample was stirred with a magnetic flea in a test tube, gassed with N_2, and then sealed for 15 h at room temperature. Base-soluble material was collected by centrifuging the preparation at 1,000*g* (5 min). The supernatant plus one distilled H_2O wash of the pellet was neutralized by dropwise addition of acetic acid. The slightly acid solution (pH 5) was held at 4°C for 4 h during which time a flocculent precipitate

Table II. *Purification of Xylanase*

Activity expressed as μg of xylose-reducing units released from larch xylan/h.

	Protein	Total Activity	Specific Activity	Yield of Activity
	mg			%
Desalted enzyme (post Bio-gel P-10)	112.0	58,240	0.52	100
Post SP-Sephadex (Fig. 1, peak D)	0.84	8,306	9.96	14.3
Post Sephadex G-100	0.310	5,425	17.50	9.3

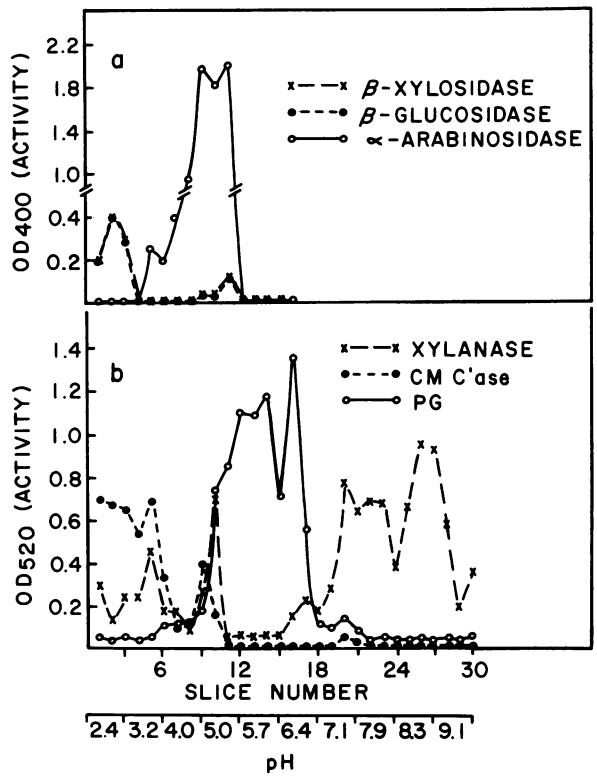

FIG. 2. A 200-μg sample of desalted Cellulysin was polymerized together with 2% (w/v) Bio-lyte 3/10 into a 6.2% (w/v) acrylamide gel contained in a 4- × 120-mm glass tube. Following isoelectric focusing, the gel was sliced into 3-mm segments which were eluted separately with 100 mM sodium acetate, pH 5.0. Eluates were assayed for (a) α-arabinosidase, β-glucosidase, and β-xylosidase and (b) PG and CMC- and xylan-hydrolyzing activities. A duplicate gel was sliced into 10-mm segments which were eluted with water. The pH of these eluates was measured to indicate the shape of the gel pH gradient.

formed. This material was collected by centrifugation, washed twice in distilled H_2O, and lyophilized.

Isoelectric Focusing. Bio-gel P-10 desalted Cellulysin was subjected to preparative isoelectric focusing using a Bio-Rad electrofocusing apparatus (model 1415). The procedures used were those of Radola (22) with minor modification. Briefly, 5 g of previously washed Bio-lyte electrofocusing gel (Bio-Rad) was suspended in 100 ml of aqueous solution containing 5 mg protein and 4% (w/v) Ampholyte (Bio-Rad). The pH gradient was designed to extend from 5 to 9 and consisted of 1.6% (w/v) each of Bio-lyte 5/7 and 7/9 and 0.8% (w/v) Bio-lyte 3-10. The gel slurry was then poured into an 11- × 30-cm glass tray and the excess water removed by evaporation. (Final weight equaled 65% that of the original slurry.) Electrode solutions were 1 M phosphoric acid (anode) and 1 M NaOH (cathode). A MRA (model 158) power supply equipped

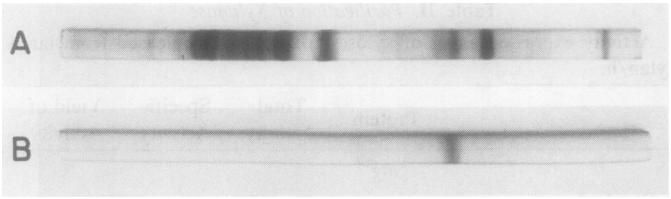

FIG. 3. Samples of (A) desalted Cellulysin, 200 μg, and (B) Sephadex G-100-purified xylanase, 70 μg, were polymerized into 6.2% (w/v) acrylamide gels and subjected to isoelectric focusing in a pH gradient from, nominally, 3 to 10. After focusing, gels were stained for protein using Coomassie Brilliant Blue R-250. Gels were destained in ethanol:acetic acid:water (5:2:12). Gels are oriented with the acidic end to the left.

with a constant power option was used to focus the plate at 15 w for 16 h. Typical voltages began at 200 v and reached 1,250 v at a peak current of 12 mamp. A temperature of 2°C was maintained at the plate by a Haake cooling apparatus (model F4391). After focusing, the gel was sectioned with a Bio-Rad gel divider. The sectioned gel was eluted with 50 mM sodium acetate (pH 5.0) and assayed for xylan and CMC hydrolyzing activity.

Analytical isoelectric focusing of the desalted Cellulysin and of xylanase-containing preparations at various stages of purification was accomplished using a modified version of the technique of Wrigley (29). Acrylamide gels (6.2% w/v) of 2 ml volume were polymerized in glass tubes (4 × 120 mm). Protein-containing preparations and ampholytes (Bio-lyte 3/10 at a final concentration of 2% w/v) were added to the acrylamide solution and polymerization was initiated by addition of ammonium persulfate to a concentration of 0.05% w/v. Anode and cathode buffers were 0.06 N H_2SO_4 and 0.06 N NaOH, respectively. Focusing was initiated with the power supply (Isco model 490) in the constant current mode and 2 mamp/gel were applied. When the overall potential reached 300 v, the power supply was set to 300 v constant voltage and the system was allowed to equilibrate.

Duplicate samples of protein were focused. One gel was stained for protein using Coomassie Brilliant Blue R-250 (0.1%) in (27:10:63) isopropanol, acetic acid, and 0.5% cupric sulfate in water (21). The second gel was frozen and sliced into 3-mm segments. Protein was eluted from these segments by placing them in tubes containing 1 ml of 100 mM sodium acetate (pH 5.0) and incubating overnight at 5°C. Enzyme activities were measured by assaying aliquots from these solutions. A third gel, containing no protein, was focused and sliced as described. These slices were eluted in tubes containing 2 ml of water. The pH of each segment was measured using a Beckman Expandomatic pH meter.

Analytical Procedures. Neutral sugar compositions of samples were determined according to the technique of Albersheim *et al.* (3). Glycosidic linkage compositions were determined by gas chromatographic analysis following methylation of samples according to the method of Hakomori (11) as modified by Sandford and Conrad (23). GC was performed as described by Ahmed and Labavitch (2).

Uronic acid concentrations were determined according to the method of Blumenkrantz and Asboe-Hansen (6). Protein concentrations were determined according to Lowry *et al.* (17) using BSA as a standard.

RESULTS AND DISCUSSION

A substantial amount of salt and low mol wt carbohydrate was eliminated from Cellulysin by gel filtration on Bio-gel P-10. Protein in the P-10 void (located by *A* at 280 nm) was lyophilized, dissolved (112 mg) in 5 ml 20 mM sodium acetate (pH 4.0), and applied to a column (15- × 120-mm gel bed) of the cation-exchanger SP-Sephadex that had been equilibrated in the same buffer. The column was eluted with 100 ml of pH 4.0 acetate and

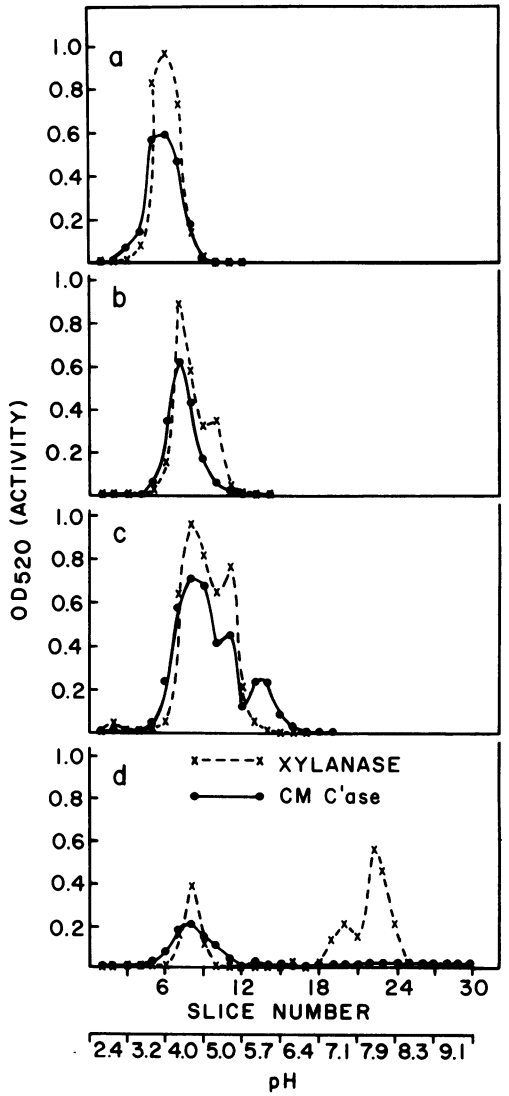

FIG. 4. Peaks of xylanase activity from SP-Sephadex chromatography (Fig. 1, peaks A, B, C, and D) were pooled separately and concentrated and desalted by ultrafiltration through a UM-2 membrane (mol wt cut-off of 2,000). Each concentrate (about 50 μg protein) was polymerized into a 6.2% (w/v) acrylamide gel with pH 3 to 10 ampholytes and subjected to isoelectric focusing. Focused gels were sliced into 3-mm segments which were eluted into 100 mM sodium acetate, pH 5.0. Eluates were assayed for xylan- and CMC-hydrolyzing activity. a, Peak A (Fig. 1); b, peak B; c, peak C; and d, peak D. A blank gel (ampholytes, no protein) was focused to determine the pH gradient.

then with a linear gradient prepared by mixing 400 ml each of 20 mM acetate and 20 mM acetate containing 500 mM NaCl. The bulk of the protein eluted in the void volume or in peaks that emerged at low ionic strength. These protein peaks were coincident with peaks (Fig. 1; A, B, and C) of CMC- and xylan-hydrolyzing activity. An additional peak of xylanase was eluted when the NaCl concentration of the buffer was increased beyond 0.2 M (Fig. 1, peak D). This peak of activity represented only a small portion of the original xylanase and very little protein. It was, however, free of CMC-hydrolase and most of the other carbohydrate-degrading enzymes present in crude Cellulysin (Table I). SP-Sephadex-purified xylanase showed a 19-fold increase in specific activity relative to the crude preparation (Table II) and had much less β-xylosidase activity than was present in the other peaks of xylanase from SP-Sephadex (data not shown). The xylanase-con-

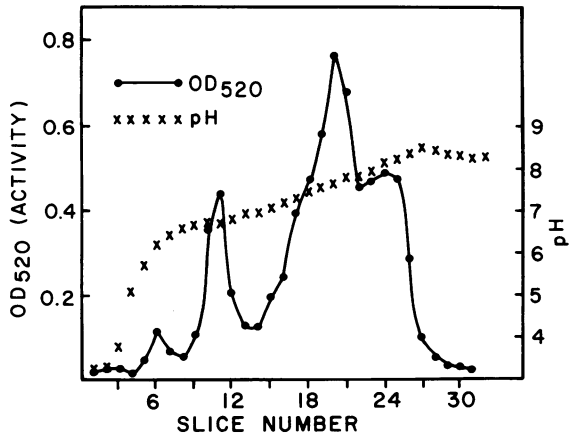

FIG. 5. Xylanase purified by ion-exchange and Sephadex G-100 gel filtration was concentrated and desalted by ultrafiltration through a UM-2 membrane. Protein (50 μg) was polymerized into a 6.2% (w/v) acrylamide gel together with pH 6 to 8 and 7 to 9 ampholytes. The gel was subjected to isoelectric focusing and then sliced into 3-mm segments which were eluted into 100 mM acetate, pH 5.0. Eluates were assayed for xylanase. The pH gradient was determined from water eluates of 3-mm segments sliced from a focused blank gel.

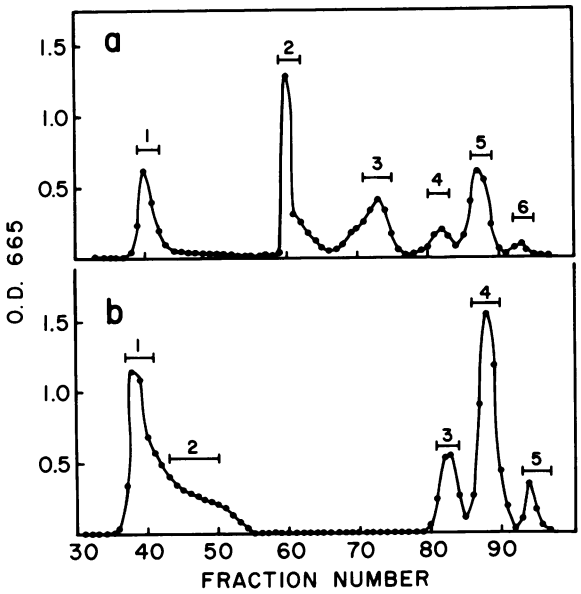

FIG. 6. Bio-gel P-2 column chromatography of products resulting from xylanase treatment of xylan substrates. The column (1.5- × 110-cm gel bed) was operated at 50°C and eluted with water (5). Fractions of 2 ml were collected and assayed for pentose with the orcinol reagent (9). Pooled fractions (indicated by numbers) were lyophilized and analyzed colorimetrically and by gas chromatography as described in "Materials and Methods." a, Digestion products from larch xylan; b, digestion products from pear wall hemicellulosic xylan.

taining fractions from the final peak were pooled, concentrated to 2 ml by dialysis against Carbowax 20,000, and dialyzed against 50 mM acetate (pH 5.2). The dialyzed concentrate was applied to a column of Sephadex G-100 (2.4- × 90-cm gel bed) that had been equilibrated in 50 mM acetate. The column was eluted with pH 5.2 acetate. This xylanase was eluted from the column just before the included volume indicating its small size (Table I, β-1,4-xylanase II). Xylanase-containing fractions were pooled and frozen. The specific activity of the G-100 purified xylanase was increased another 2-fold over that of the preparation from SP-Sephadex. The preparation represented only a small fraction of the protein and xylanase present in the crude protein preparation and was free of the other carbohydrate-degrading enzymes present initially, including β-xylosidase (based on 16-h incubations with the substrates listed in "Materials and Methods").

The efficiency of purification of the xylanase was followed by subjecting protein samples from each column chromatographic step to isoelectric focusing in a broad pH gradient (about 3 to 10). Gels were stained for protein and proteins eluted from duplicate gels were assayed for the more significant enzyme activities (xylanase, PG, CMC-hydrolase, α-arabinosidase, β-galactosidase, and β-xylosidase) identified in Cellulysin. The data (Figs. 2 and 3A) clearly indicate that most of the protein in the Bio-gel P-10 desalted preparation has a pI below pH 6.0 and, of the enzyme activities tested, the only carbohydrase present in the alkaline part of the gel is a xylanase.

The separate peaks of xylanase activity identified following SP-Sephadex ion exchange chromatography (Fig. 1) were concentrated, desalted by ultrafiltration through a membrane with a mol wt cut-off of 2,000 (Amicon UM-2) and subjected to focusing in a pH 3 to 10 gradient. Xylan- and CMC-hydrolyzing activities are largely coincident in samples eluted from the ion-exchanger at low ionic strength and it is apparent that there are multiple species of these enzymes (Fig. 4, a–c). In one case, isoelectric focusing of these weaker anionic proteins appears to resolve activity against CMC that is free from xylanase (Fig. 4c). The xylanase peak which associated with SP-Sephadex most strongly is resolved into an acidic peak (with overlapping CMC-hydrolase, similar to peaks seen in Fig. 4, a–c) and a pair of peaks (pI values of 7.1 and 7.9) which are free of CMC-hydrolase activity even after extended incubations (16 h at 37°C).

The xylanase purified using SP-Sephadex and Sephadex G-100 was subjected to isoelectric focusing in a pH 3 to 10 gradient. As before, the xylanase activity resolved in the alkaline end of the gel, being coincident with a single sharp, and a series of faint, Coomassie Blue-staining bands. One faint Coomassie-positive band not coincident with xylanase was observed. The purified xylanase was also subjected to focusing in a pH 6 to 9 gradient (Fig. 5). The enhanced resolution afforded by this gradient indicated the presence of at least four xylanase isomers with pI values ranging from 6.2 to 8.1.

The combined isoelectric focusing data suggested an alternative to the column chromatography purification scheme described above. Accordingly, desalted Cellulysin was subjected to preparative isoelectric focusing in a pH 5 to 9 gradient as described in "Materials and Methods." Xylanase free of contaminating activities focused in a broad band in the region of the gel bed between pH 6.5 and 8.0. Analytical isoelectric focusing of protein from this region of the gel revealed a family of xylanase isomers similar to that seen for the xylanase purified by column chromatography (data not shown).

Characteristics of Purified Xylanase. Purified xylanase was dialyzed against distilled H_2O and adjusted to a range of hydrogen ion concentrations (pH 3.0–7.0) and assayed. Optimum activity was measured at pH 5.0 with activity falling off to less than 50% of maximum only when the pH was below 3.5 or above 7.0.

Heat stability of xylanase was determined by treating the enzyme for 30 min at a range of temperatures and then assaying at 37°C and pH 5.0. Activity fell off rapidly above 45°C. The enzyme was completely inactive after 30 min at 65°C. There were no differences in the activity of preparations held at 22°C or 45°C.

The nature of xylanase action on its substrate was assessed by analyzing the carbohydrate products following a prolonged incubation period. The xylan used for routine assay is a linear, 1,4-linked polymer bearing occasional side groups consisting of single arabinosyl and glucuronosyl residues (data not shown). It is not readily water soluble. In order to prepare a 5 mg/ml solution the xylan was first dissolved in 0.5 N NaOH and then adjusted to pH

5.0 with HCl.

Purified xylanase (activity sufficient to generate 500 xylose reducing equivalents/h) was incubated for 16 h with 1 ml (5 mg) of larch xylan. Following incubation the preparation was centrifuged to remove insoluble material. The supernatant was applied to a column of Bio-gel P-2 (120- × 1.4-cm gel bed) and eluted with water (5). Peaks representing oligomeric digestion products and largely undigested material were located colorimetrically (9) (Fig. 6a). Individual oligomer peaks were pooled and lyophilized. The sugar and glycosidic linkage composition of these products was determined as described in "Materials and Methods." Mono-, di-, and trisaccharides, apparently from the xylan backbone, were identified by co-elution (with glucose, maltose, and raffinose, respectively) from the P-2 column and by methylation analysis (data not shown). In addition, there were larger oligomeric peaks which contained arabinose as well as xylose (Fig. 6a, peaks 2 and 3). The methylation analysis (data not shown) indicated that the arabinose was present as terminal residues (covalently linked only through C-1). Apparently the xylanase can cleave the substituted 1,4-xylan backbone at glycosidic linkages near arabinose-substituted xylosyl residues.

Digestion of Pear Hemicellulose. A precipitate forms when the 4.4 N NaOH-soluble portion of Bartlett pear cell walls is neutralized. Analysis of this material has shown it to have a 1,4-linked xylan backbone (1); a similar polymer, bearing glucuronosyl substituents, is present in the walls of the 'Conference' pear (8).

The precipitated pear wall xylan was dissolved, enzyme-treated, and chromatographed as for the larch xylan substrate. In this case, the predominant digestion products are mono-, di-, and trimers containing xylose (Fig. 6b, peaks 3–5). These products contain no uronosyl residues; uronic acids are restricted to the larger-sized carbohydrates which chromatograph at the Bio-gel P-2 column void volume (Fig. 6b, peaks 1 and 2). It is not clear why the xylanase hydrolyzes the polymer backbone which bears arabinosyl side groups but not one containing uronosyl (presumably glucuronosyl; 8) residues.

The work described above indicates that several species of β-1,4-xylanase which are free of CMC-hydrolyzing activity are produced by *Trichoderma viride* under the culture conditions used to produce Cellulysin. Collectively, these xylanases can act on larch xylan and pear xylan to generate products which are primarily oligomeric. For this reason, we conclude that these xylanases are endoglycanases. However, because some monosaccharide xylose is produced (Fig. 6, a and b) and because a family of xylanase activities was identified by the production of reducing sugars (Fig. 5), it is possible that some exoxylanase is included in the purified preparation.

The column chromatographic purification scheme described indicates only a 35-fold increase in specific activity in spite of a great decrease in total protein (Table II). This rather small increase in specific activity is probably a minimum value; the 'true' xylanase activity of impure preparations being obscured by the presence of β-xylosidase which is completely removed only after gel filtration on Sephadex G-100. The xylosidase could degrade oligomers produced by xylanase to monosaccharides and thus increase the yield of reducing sugars from xylan. Only the final xylanase specific activity (post Sephadex G-100) can be viewed as accurate. It would have been possible to clarify this point, as well as the question of the presence of endo- and exoxylanases, had carboxymethylxylan been synthesized (24) and used in viscometric and reducing sugar assays. In light of the fact that the purified xylanase degraded the xylan of interest (pear xylan), this point was not pursued.

The xylanase described here is similar in many respects to xylanases purified from *T. viride* culture filtrates by more elaborate schemes (13, 28). Toda *et al.* (28) have described purification of a xylanase of mol wt 16,000 that is free from cellulase activity. Their scheme also identified several coincident peaks of xylanase and cellulase which they assumed were dual activities of single species of protein. Similar suggestions have been made by others (14). We have used a more powerful means of resolution than they (isoelectric focusing) and were able to identify only a minor amount of CMC-hydrolyzing activity free of xylanase (Fig. 4c). Hashimoto *et al.* (13) have purified and crystallized a xylanase from *T. viride*. Their data did not indicate whether the crystalline protein had cellulase activity.

Cellulysin is an enzyme preparation that has been widely used in research. This use is often in the production of protoplasts and it is clear from the assortment of enzymes it contains (Table I; Fig. 2) that it is well suited to the task. Some caution must be exercised, however, if protoplasts are to be used for work related to the behavior of the plant cell surface because glycosidases present could alter exposed glycoproteins. A potentially more significant problem is the fact (data not shown) that Cellulysin contains a protease of the azocaseinase type. Similar concerns have been raised by Harrison and Black (12) who demonstrated, by two-dimensional electrophoresis, a series of proteins (of undefined activity) in the 'cellulase' they used to make protoplasts from *Digitaria sanguinalis*. Conclusions based on the presumption that Cellulysin acts only on β-1,4-glucans are clearly suspect.

Homo- and heteroxylans are of widespread occurrence in plants (1, 4, 7, 8, 18), especially in woody tissues. Given the widespread interest in cell wall structure from both the biochemical and technological points of view, it is important to have specific tools for analysis of polymer structure. Either of the simple purification schemes described above can provide, from a readily available source, a xylanase that is homogeneous with respect to cell wall substrate specificity.

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