Solubilization of Peroxidase Activity from Cotton Cell Walls by Endopolygalacturonases

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ABSTRACT

Isolated cell walls from *Gossypium hirsutum* and *G. barbadense* contained peroxidase activity. Incubation of these wall fractions with purified endopolygalacturonases from either *Verticillium albo-atrum* or *Fusarium oxysporum* f. sp. *lycopersici* resulted in solubilization of a protein fraction that contained peroxidase activity. Exopolygalacturonase purified from *V. albo-atrum* did not solubilize the peroxidase activity.

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Additional key words: pathogenesis, disease resistance.

Proteins and enzymes associated with plant cell walls apparently are involved in normal plant growth responses, and participate in host-parasite interactions during pathogenesis (1, 6, 9). When potato tuber slices were incubated in a solution containing a purified endopectate-*trans*-eliminase produced by *Erwinia carotovora,* several enzymes were released into the incubation medium (10). Similarly peroxidases bound to soybean cell walls were released when the walls were treated with a culture filtrate of *Sclerotium rolfsii* that contained wall-hydrolyzing enzymes (2, 4). Because both *trans*-eliminative and hydrolytic endopectate depolymerases cause disease symptoms to appear when applied to host tissue (3, 7) possibly through the generation of peroxides (6, 7), we have investigated the effects of endopolygalacturonases (endoPG's) on peroxidase activity associated with cotton cell wall fractions.

MATERIALS AND METHODS.—Exopolygalacturonase (exoPG) and endoPG (EC 3.2.1.15) from *Verticillium albo-atrum* Reinke & Berth., and endoPG from *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. (Race 1) were purified as previously described (8, 11). Seeds of *Gossypium hirsutum* 'M-8', 'Acala SJ-1', and 'Coker 310', and *G. barbadense* 'Seabrook-Sea Island' were germinated and grown 8 days in darkness at 26 to 28 C on Jiffy-Mix (Jiffy Products of America, West Chicago, Ill.). Hypocotyl cell walls were prepared according to Barnett (2) by homogenizing hypocotyl sections in cold 0.1 M sodium phosphate, pH 7.4, containing 1% *n*-octanol, then consecutively washing the resultant brei with several liters of ice-cold distilled water, cold 2 N sodium chloride in 0.1 M sodium phosphate, pH 7.4, and ice water. Aliquots containing approximately 50 mg of the resulting cell wall material were suspended in 10 ml of 10 mM sodium phosphate and incubated with 1 ml of pectic enzyme added in 20 mM sodium phosphate. The pH's of the incubation mixtures were 6.0 for the Verticillium enzymes and 5.0 for the Fusarium enzyme. The endoPG's were added to a final activity of 200 relative viscometric units [RVu, as described in (7)] per ml of incubation mixture; the exoPG was added to a final activity of 20 RVu per ml. Suspensions were shaken for 3 hours at 30 C, filtered, and the filtrates were assayed for solubilized proteins and peroxidase. Controls received 1 ml of 20 mM sodium phosphate without enzyme.

Protein was determined according to Hartree (5) using bovine serum albumin as a standard. Barnett's guaiacol oxidation assay for peroxidase (2) was used to measure soluble and particulate peroxidase. Absorbance of the oxidized phenol was read at 470 nm in a Beckman Model B spectrophotometer. Assays for particulate peroxidase were carried out in the absence of direct light. Peroxidase activity was converted to μmoles hydrogen peroxide reduced per minute using published extinction

TABLE 1. Release of protein and peroxidase activity from cotton cell walls after 3 hours of incubation with polygalacturonases

	Cultivars used as cell wall sources			
	Seabrook-Sea Island	M-8	Acala SJ-1	Coker 310
Total peroxidase associated with the cell walls[a]	240	220	260	140
Verticillium endoPG:				
solubilized peroxidase[a]	110	29	160	55
solubilized protein[b]	3.7	3.8	8.0	7.2
Fusarium endoPG:				
solubilized peroxidase[a]	82	8.3	74	18
solubilized protein[b]	7.9	4.0	8.5	3.8
Verticillium exoPG:				
solubilized peroxidase[a]	0	0	0	0
solubilized protein[b]	0	0	0	0
Control (20 mM phosphate):				
solubilized peroxidase[a]	0	0	0	0

[a]Nanomoles hydrogen peroxide reduced per minute per mg cell wall.
[b]Micrograms protein per mg cell wall.

coefficients for tetraguaiacol (2). Peroxidatic oxidation of guaiacol was verified by the absence of oxidation when hydrogen peroxide was omitted from the reaction mixtures. The purified pectic enzyme solutions used in these studies did not contain peroxidase activity.

RESULTS AND DISCUSSION.—Cell wall suspensions of all four cotton cultivars contained peroxidase activity that was not removed by washing the cell walls with water, buffer, or a high concentration of sodium chloride (Table 1). This apparently bound peroxidase activity was readily solubilized by incubation of the walls with the endoPG from either *V. albo-atrum* or *F. oxysporum* f. sp. *lycopersici,* but not by incubation with an exoPG produced by *V. albo-atrum* (Table 1). The amount of exoPG used in these studies contained the same potential bond-hydrolyzing activity as the endoPG's; the lower viscosity-reducing activity of the exoPG was due to its terminal mode of hydrolysis (8).

Our results, and the data of Barnett (2) and Stephens and Wood (10), provide unambiguous evidence that pectic depolymerases can cause the release of bound peroxidases from plant cell walls. These results also indicate that solubilization of peroxidases can be achieved with endo-acting pectic depolymerases, but not with an exo-acting enzyme. In view of the evidence that endo-acting pectic enzymes, but not exoPG, will cause disease symptoms when applied to host tissues (3, 7), solubilization of host cell wall peroxidases, and their interaction with native substrates, may be critical events in the biochemistry of pathogenesis.

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