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Antifungal Hydrolases in Pea Tissue[1]

II. INHIBITION OF FUNGAL GROWTH BY COMBINATIONS OF CHITINASE AND β-1,3-GLUCANASE

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ABSTRACT

Chitinase and β-1,3-glucanase purified from pea pods acted synergistically in the degradation of fungal cell walls. The antifungal potential of the two enzymes was studied directly by adding protein preparations to paper discs placed on agar plates containing germinated fungal spores. Protein extracts from pea pods infected with *Fusarium solani* f.sp. *phaseoli*, which contained high activities of chitinase and β-1,3-glucanase, inhibited growth of 15 out of 18 fungi tested. Protein extracts from uninfected pea pods, which contained low activities of chitinase and β-1,3-glucanase, did not inhibit fungal growth. Purified chitinase and β-1,3-glucanase, tested individually, did not inhibit growth of most of the test fungi. Only *Trichoderma viride* was inhibited by chitinase alone, and only *Fusarium solani* f.sp. *pisi* was inhibited by β-1,3-glucanase alone. However, combinations of purified chitinase and β-1,3-glucanase inhibited all fungi tested as effectively as crude protein extracts containing the same enzyme activities. The pea pathogen, *Fusarium solani* f.sp. *pisi*, and the nonpathogen of peas, *Fusarium solani* f.sp. *phaseoli*, were similarly strongly inhibited by chitinase and β-1,3-glucanase, indicating that the differential pathogenicity of the two fungi is not due to differential sensitivity to the pea enzymes. Inhibition of fungal growth was caused by the lysis of the hyphal tips.

Biochemical research on disease resistance has two prime objectives: first, to characterize the biochemical mechanisms by which plants inhibit or destroy potential pathogens in general, and second, to establish what specific factor or factors determine the compatibility or incompatibility in particular host-pathogen interactions (5). In the present communication, we do not address the second theme but deal with an interesting facet of the first one. We provide direct evidence that combinations of two pathogen-induced plant proteins, chitinase and β-1,3-glucanase, strongly inhibit growth of many pathogenic fungi.

In addition to the variety of preformed and inducible secondary compounds, chitinase and β-1,3-glucanase have long been suggested to belong to the antifungal defenses of plants (1, 8, 9, 23). This is based on the following indirect evidence: First, high activities of chitinase and β-1,3-glucanase are frequently found in higher plants (8, 9), but chitinase has no known substrate in the plant itself, and the substrate for β-1,3-glucanase, callose, is usually present only in small quantities (1). However, chitin and β-1,3-glucans are important structural elements of the cell walls of many fungi (27) and may represent the natural substrate for the two plant hydrolases. Second, purified plant chitinase and β-1,3-glucanase can partially degrade isolated fungal cell walls (6, 15, 28). Third, chitinase and β-1,3-glucanase are coordinately induced by ethylene and by pathogen infections or pathogen-derived elicitors in various tissues (1, 19, 24). Fourth, a bean pathogen, *Colletotrichum lindemuthianum*, has been observed to produce proteins which inhibit plant β-1,3-glucanase (2). This may be interpreted as an evolutionary adaptation of the pathogen to one of the plant's defenses.

Since we have purified two chitinases and two β-1,3-glucanases from infected young and uninfected old pea pods, as reported in the preceding article (20), we became interested in determining

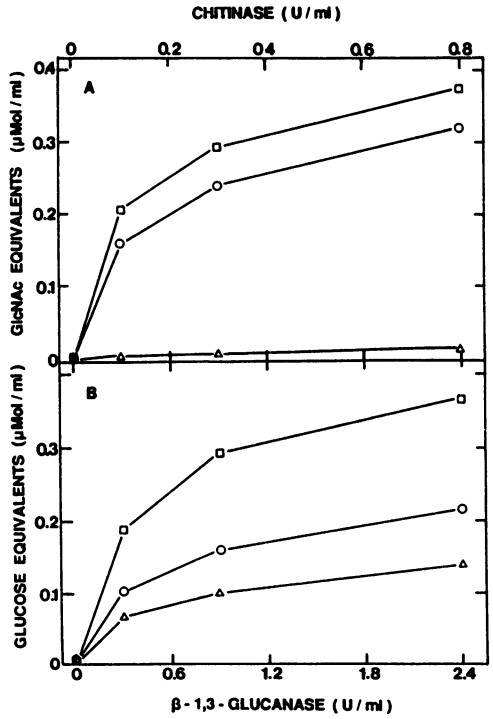

FIG. 1. Release of soluble GlcNAc oligomers (A) and of soluble reducing oligosaccharides (B) from isolated cell walls of *F. solani* f.sp. *phaseoli* by purified chitinase (○), purified β-1,3-glucanase (△) and by a combination of the two enzymes (□). Incubation was at 37°C for 2 h. The enzymes were purified from uninfected old pea pods. The β-1,3-glucanase purified was of the form G1; chitinase was a mixture of the forms Ch1 and Ch2 (20). Chitinase activity was determined by the colorimetric assay (17).

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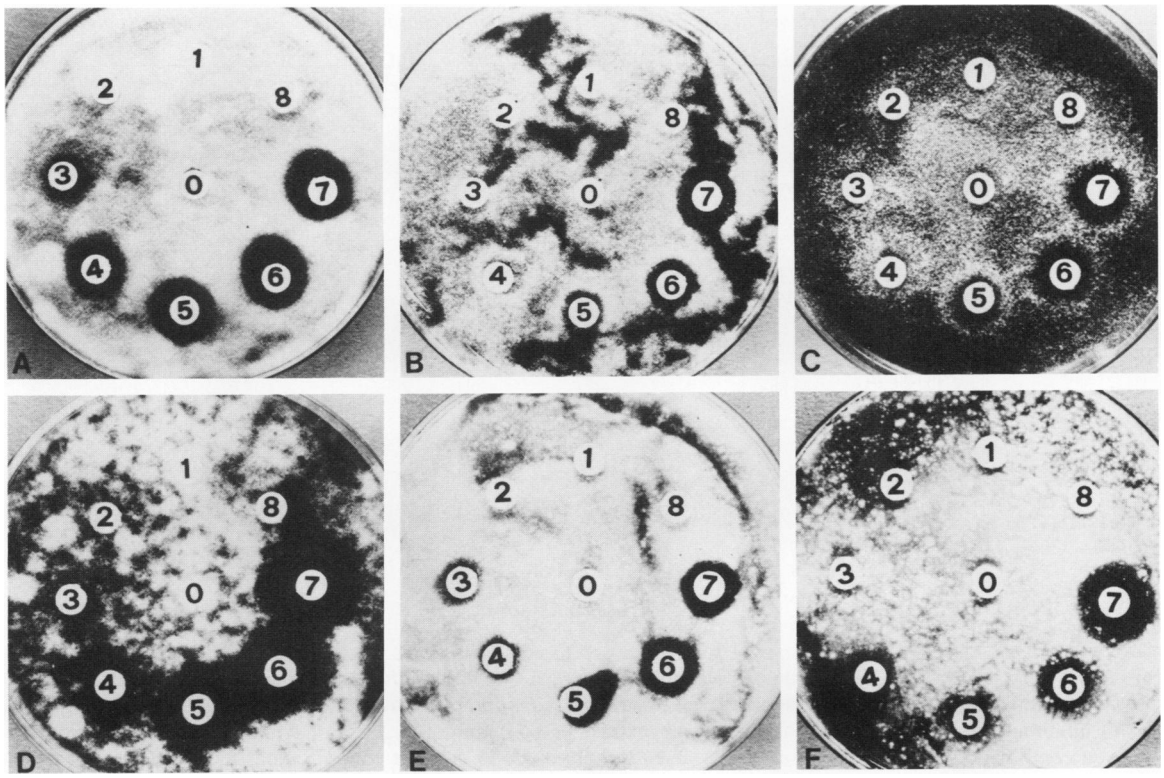

FIG. 2. Effect of protein preparations on growth of various fungi. Spores of the fungi were germinated on agar plates for 20 h. Subsequently, filter discs were placed on the agar, and 40 μL of the following protein preparations were pipetted on the discs: 1, water; 2, crude protein extract (1×) from untreated immature pea pods (containing 0.09 μg Ch1-equivalents and 0.26 μg G1-equivalents); 3, crude protein extract (1×) from pea pods incubated with sterile water for 24 h (containing 0.24 μg Ch1-equivalents and 0.37 μg G1-equivalents); 4, 5, 6, and 7, crude protein extracts (0.5×, 1×, 2×, and 5×, respectively) from pea pods incubated with *F. solani* f.sp. *phaseoli* for 24 h (the extract 1× contained 1.3 μg Ch1-equivalents and 2.6 μg G1-equivalents); 8, crude protein extract (1×) from infected pea pods, boiled for 10 min before application; 0, Cyt *c* (1 mg mL^{-1}). The amount of protein per ml in the extracts 1× was 6 mg mL^{-1} and corresponded to the amount of protein extracted from 1 g fresh weight of the tissue. A, *F. oxysporum* f.sp. *lycopersici*; B, *M. fructigena*; C, *T. basicola*; D. *A. solani*; E, *A. bombacina*; F, *A. niger*.

if the postulated antifungal activity of chitinase and β-1,3-glucanase could be demonstrated directly. In this article, we demonstrate that combinations of purified chitinase and β-1,3-glucanase strongly inhibit growth of a number of potentially pathogenic fungi.

MATERIALS AND METHODS

Biological Material. Pods of *Pisum sativum* L. cv "Dot" (an Alaska-type variety) were obtained as described (20). *Fusarium solani* f.sp. *phaseoli* (strain W8, American Type Culture Collection, ATCC 38135) and *Fusarium solani* f.sp. *pisi* (ATCC 38136), were gifts from Dr. L. A. Hadwiger, Washington State University, Pullman. Different pathotypes of *Ascochyta pisi* were kindly provided by Dr. P. Matthews, Norwich, and by Dr. M. Allard, Versailles. The following fungi were provided by Dr. F. J. Schwinn, Basel, and by Dr. U. Gisi, Witterswil: *Alternaria solani*, *Aspergillus niger*, *Athelia bombacina*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Colletotrichum lagenarium*, *Drechslera sativa*, *Fusarium culmorum*, *Fusarium oxysporum* f.sp. *lycopersici*, *Monilinia fructigena*, *Penicillium digitatum*, *Phoma Betae*, *Pythium aphanidermatum*, *Pythium ultimum*, and *Thielaviopsis basicola*. *Phytophthora cactorum* was a gift of S. Jaunin, Basel. All fungi were cultivated on malt extract agar with the following exceptions: *Ascochyta pisi* was grown on modified Coon's Agar (14), *C. lagenarium* on the medium of Marthur *et al.*, (18), and the Oomycetes *P. cactorum*, *P. aphanidermatum*, and *P. ultimum* on V8-agar.

Preparation of Plant Extracts and of Purified Enzymes. The treatment of immature pea pods with sterile water or a spore suspension of *F. solani* f.sp. *phaseoli* as well as the incubation and extraction conditions are described in the preceding paper (20). The protein pellet obtained from crude extracts after ammonium sulfate precipitation (95% saturation) was redissolved in water and extensively dialyzed against water. For the test of antifungal activity, the dialyzed extracts were filtered through a 0.22 μm membrane filter, lyophilized and taken up in sterile water just before use. The purification of the different forms of chitinase and β-1,3-glucanase, the corresponding enzyme assays, and the protein measurements were performed as described (20).

Isolation and Enzymatic Degradation of Isolated Fungal Cell Walls. *F. solani*, f.sp. *phaseoli* and f.sp. *pisi*, were grown in liquid culture (13) on a reciprocal shaker set at 60 rpm at room temperature. Fungal mycelium was harvested after 28 h of incubation, and fungal cell walls were isolated as described (6), except that the mycelium was homogenized in water instead of buffer. The isolated fungal cell walls were repeatedly sonicated and washed until no cytoplasmic contamination could be detected by light microscopy. The final cell wall suspension was sonicated again just prior to use for degradation studies.

The enzymic degradation of the isolated cell walls was performed in a reaction mixture containing 10 μmol sodium acetate buffer (pH 5.5), 1.2 mg isolated cell walls, and enzyme solution in a total volume of 1 mL. The reaction mixture was incubated at 37°C on a shaking water bath for 2 h and stopped by centrifugation (5 min, 2000*g*). The release of reducing sugars was determined as described (11). The released water-soluble chitooligosaccharides were degraded to GlcNAc by the action of snail

Table I. *Inhibition of Growth of Various Fungi by Crude Protein Preparations from Infected Pea Pods and by Purified Enzymes*

Test Fungus	Sensitivity[a]	
	Crude protein preparation	Combination of Ch1 and G2[b]
F. solani f.sp. *phaseoli*	+++	+++
F. solani f.sp. *pisi*	+++	+++
F. culmorum	+++	ND
F. oxysporum f.sp. *lycopersici*	+++	ND
Ascochyta pisi, pathotype 1, 2 and 4	+++	+++
C. lagenarium	+++	ND
A. solani	+++	ND
M. fructigena	+++	ND
P. digitatum	+++	+++
A. niger	+++	+++
T. basicola	+++	+++
A. bombacina	+++	+++
T. viride	+++	+++
B. cinerea	++	ND
C. cucumerinum	+	ND
Ph. cactorum[c]	−	ND
P. ultimum[c]	−	ND
P. aphanidermatum[c]	−	ND

[a] The scale was: +++, inhibition by a protein extract "1x" (*cf.* Fig. 3), *i.e.* at a concentration (amount per mL) equivalent to the amount per g infected tissue; ++, inhibition by a twice concentrated protein extract; +, inhibition by a five times concentrated protein extract; −, no inhibition. ND, not determined. [b] Test using a combination of purified chitinase Ch1 and β-1,3-glucanase G2 (20) at concentrations (activities per ml) equivalent to their activities per g infected tissue. [c] Tested against radial growth of fungal colony.

gut enzyme (Cytohelicase, IBF, Clichy, France), and the GlcNac was assayed colorimetrically (6). All assays were run in triplicate and showed mean standard deviations of less than 10%. The data were corrected using enzyme blanks, substrate blanks, and internal standards of glucose and GlcNac, respectively.

Assay of Antifungal Activity. All manipulations were carried out under sterile conditions. Fungal spores were harvested from well sporulating colonies and suspended in sterile water. The concentrations of the spore suspensions were determined in a hemacytometer and adjusted to 1.0 to 2.5 × 10^6 spores per ml, depending on the fungus to be tested. The freshly prepared suspensions (0.5 and 2.0 mL for plates with a diameter of 90 and 150 mm, respectively) were plated out on Petri dishes containing the nutrient agar used for maintenance of the test fungus. To allow for spore germination and initial vegetative growth, the plates were incubated for 20 to 24 h at room temperature. At this time, sterile filter paper discs (4 mm diameter) were laid on the agar surface, and 40 μL of the solutions to be tested were applied to the discs. The plates were further incubated at room temperature and photographed 24 to 72 h after the onset of treatment. All test solutions were filtered through a 0.22 μm membrane filter prior to application.

To evaluate effects of the test solutions on fungal growth, samples adjacent to the filter discs were taken at intervals from 0 to 7 h after application of the test solutions. The samples were squashed, stained with lactophenol cotton blue, observed with a light microscope (Wild, M20), and photographed.

Antifungal activity against Oomycetes was tested by applying the extracts to wells punched into the agar in front of the growing fungus (26).

RESULTS

Degradation of Isolated Fungal Cell Walls by Chitinase and β-1,3-glucanase. Cell walls isolated from *Fusarium solani* f.sp. *phaseoli*, a nonpathogen of pea, were incubated with preparations of purified chitinase and β-1,3-glucanase and with their combinations. The results of a representative experiment are shown in Figure 1. When assayed individually, both enzymes released soluble reducing oligosaccharides from the cell walls. Chitinase, but not β-1,3-glucanase, released GlcNAc oligosaccharides from the cell walls. However, combinations of chitinase and β-1,3-glucanase released more GlcNAc oligomers than chitinase alone, indicating a synergistic effect of β-1,3-glucanase on the degradation of cell wall chitin by chitinase. The amount of soluble product formed at the highest enzyme concentration corresponded to about 10% of the dry weight of the cell walls.

The cell walls of *F. solani* f.sp. *pisi*, a pathogen of peas, were partially degraded, but the amount of reducing sugars and GlcNAc oligomers released was about 30% lower per mg cell wall material than with the walls of *F. solani* f.sp. *phaseoli* (data not shown). The preparations from *F. solani* f.sp. *pisi*, which formed many more microconidia in liquid culture, contained a higher proportion of spore walls. Isolated spore walls were found to be more resistant to enzymic attack (our unpublished observations).

Inhibition of Fungal Growth by Protein Preparations from Pea Pods. Protein extracts were prepared from immature pea pods by ammonium sulfate precipitation followed by dialysis against water. To test for antifungal activity, aliquots of the sterile filtered protein preparations were added to filter discs on agar plates containing germinating fungal spores. (The concentration of protein solution applied to the discs was adjusted to the mean concentration of proteins in the tissue, or to multiples thereof, to allow for easy comparison.) In the following 24 h, distinct inhibition zones developed around the filters treated with the extracts from infected tissue (Fig. 2). Out of 15 fungal strains (with a chitin-glucan cell wall) tested, 13 were susceptible to an extract with a protein concentration equivalent to the concentration of soluble protein in the original tissue (Table I). The growth of the remaining two strains was inhibited only by two- and five-times concentrated extracts. Extracts from untreated, immature pods did not inhibit fungal growth, showing that the inhibitory activity was induced in response to fungal infection. Some fungi

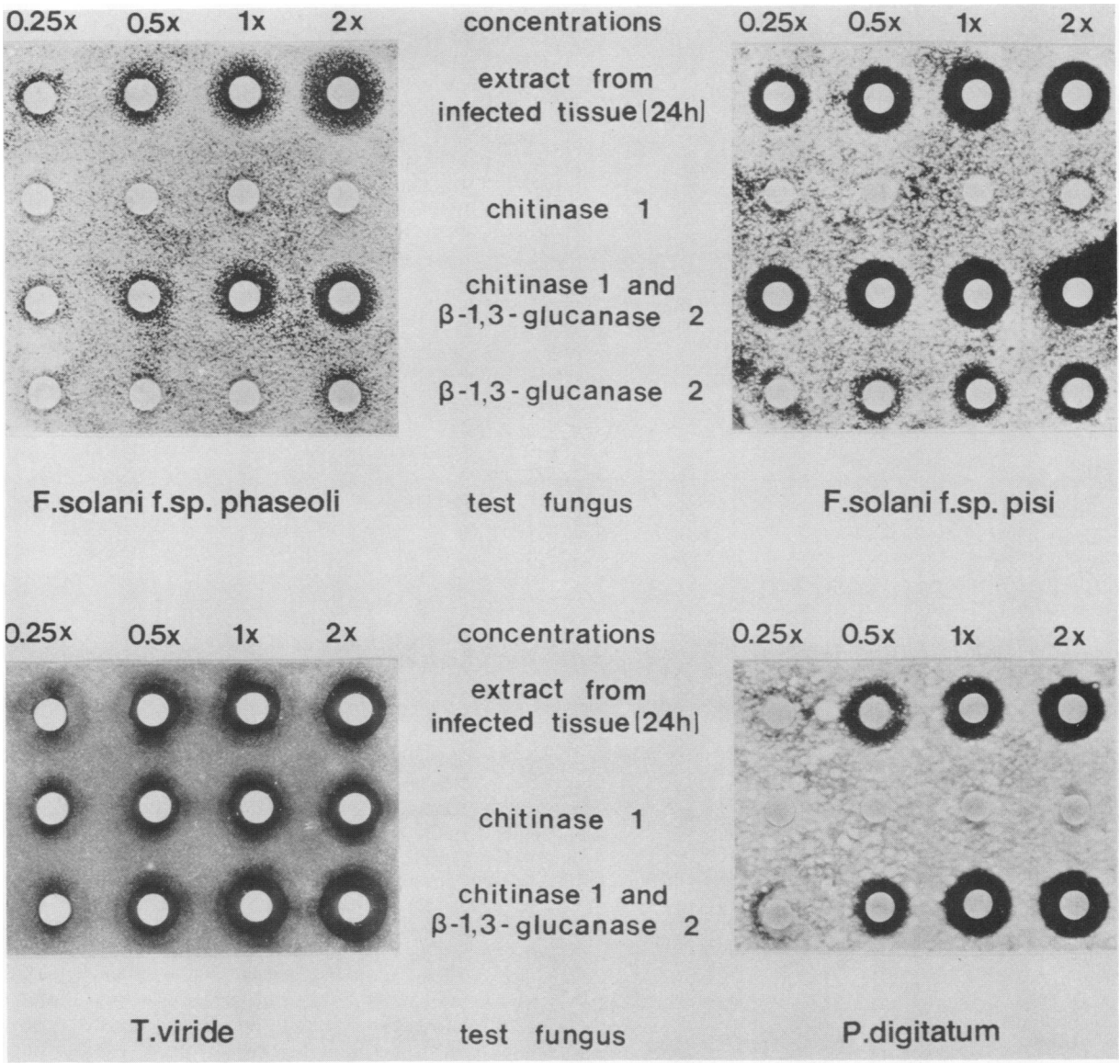

FIG. 3. Inhibition of fungal growth by crude protein preparations and by purified enzymes. Fungal spores were allowed to germinate and grow for 24 h prior to the application of the test solutions (40 μL). The discs contained either crude protein preparations from infected pea pods (top row), or chitinase Ch1 and β-1,3-glucanase G2 purified from the infected pods (20), singly (rows 2 and 4) or in combination (row 3). The amount of enzymes and protein present in the 40 μL solutions applied to the discs of the column labeled 1× were equivalent to the amount present in 40 mg fresh weight of the infected tissue, *i.e.* 1.3 μg chitinase, 2.6 μg β-1,3-glucanase, and 200 μg of protein.

were slightly inhibited by protein extracts from pea pods incubated with sterile water, indicating that the inhibitory activity was induced to some extent by wounding. Boiling of the protein preparation from infected pods for 10 min completely destroyed the inhibitory activity against all test fungi. Cyt *c*, a basic protein like chitinase and β-1,3-glucanase, did not inhibit growth of any of the test fungi. In general, there was a good correlation between the chitinase and β-1,3-glucanase activities present in an extract and its inhibitory activity on fungal growth.

Three representatives of the Oomycetes were also tested. Since these fungi did not readily form spores, they were subjected to a radial growth inhibition assay described earlier for *Trichoderma viride* (26). None of them was inhibited by any of the preparations tested.

Inhibition of Fungal Growth by Purified Chitinase and β-1,3-Glucanase. In order to examine the contribution of chitinase and β-1,3-glucanase to the observed antifungal activity of crude protein extracts, purified enzymes were tested individually or in combination with eight different fungi. The inhibitory activity of a dilution series of a crude protein preparation from infected pea pods was directly compared with a dilution series of purified chitinase and β-1,3-glucanase adjusted to the enzyme concentration present in the crude protein preparations (Fig. 3). When applied individually, chitinase was inhibitory only to *T. viride*, and β-1,3-glucanase only to *F. solani* f.sp. *pisi*. The growth of all other fungi tested with purified enzymes (Table I) was inhibited only when both enzymes were used in combination. Inhibition of fungal growth by combinations of the purified enzymes was as effective as the inhibition caused by the corresponding crude protein preparation, indicating that chitinase and β-1,3-glucanase are the main antifungal proteins in pea tissue. The growth of most of the test fungi was inhibited by chitinase and β-1,3-glucanase concentrations in the range of 10 to 30 μg mL^{-1}. Infected tissue contained 1.3 μg chitinase and 2.6 μg β-1,3-

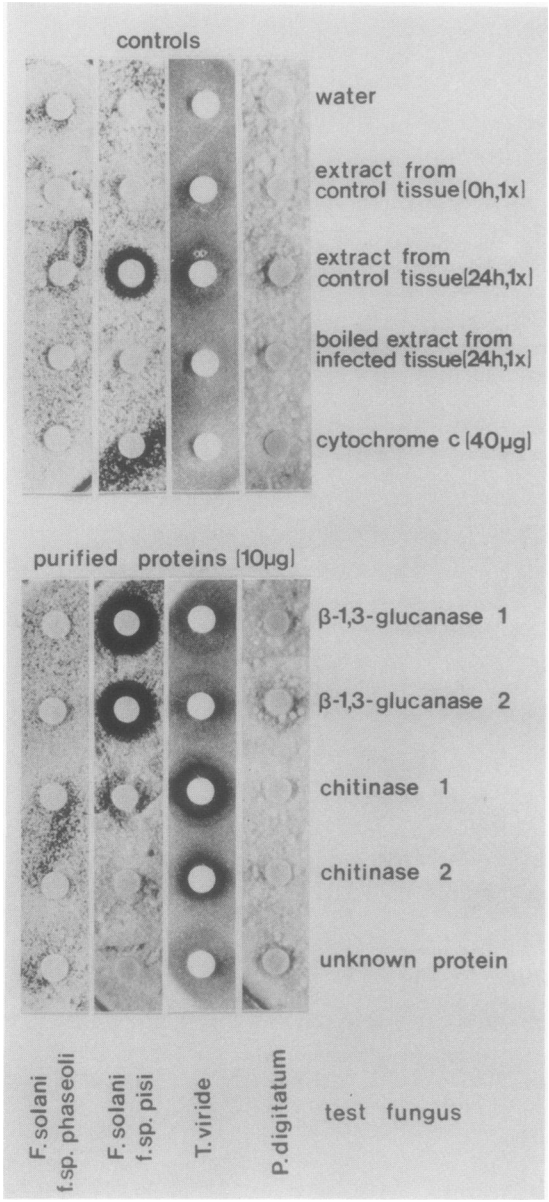

FIG. 4. Effect of crude protein preparations and of purified proteins on fungal growth. Test conditions were the same as described in Figure 3. The control extracts are described in Figure 2.

glucanase per 40 mg fresh weight, corresponding to 32 µg chitinase and 65 µg β-1,3-glucanase per mL tissue.

The most sensitive fungi were *T. viride* and *F. solani* f.sp. *pisi*, which were inhibited by protein extracts from uninfected and wounded pea pods, respectively (Fig. 4). As little as 1 µg mL^{-1} chitinase showed an inhibitory effect on *T. viride*. However, *T. viride* was the only fungus to quickly overcome this inhibition. It started to overgrow the initial inhibition zones within 24 h after application of the test solutions. In all other cases, the inhibition zones remained visible for at least 1 week.

Each of the purified proteins was also tested at a higher concentration of 10 µg per assay disc, corresponding to 250 µg ml^{-1} (Fig. 4). Both β-1,3-glucanases inhibited growth of *F. solani* f.sp. *pisi*. The chitinases Ch1 and Ch2 (20) inhibited only *T. viride*. The pathogen-induced chitinase Ch1 was slightly more inhibitory than the maturation-related chitinase Ch2. All other fungi tested were not inhibited by high concentrations of single enzymes, indicating an almost absolute requirement for both enzyme activities to cause inhibition of fungal growth. The unknown protein (20) did not inhibit any of the test fungi.

Lysis of the Hyphal Tips. An examination by light microscopy of the mycelium developing around the filter discs showed that the inhibition of fungal growth by extracts from infected tissue was caused by the lysis of the hyphal tips (Fig. 5, E and F). Lysis of the hyphal tips appeared to be the cause of growth inhibition for all fungi listed in Table I. Hyphae growing under the influence of an extract from untreated pea pods did not show any obvious growth aberrations (Fig. 5, A and B). Treatment of the fungi with purified enzymes caused the same swelling and lysis of the hyphal tips as observed with the crude protein extracts from infected tissue (Fig. 5, G and H). The lysis of the fungal tips by a combination of chitinase and β-1,3-glucanase was a fast reaction. In some instances, the fungal tips started to burst within 1 min after the direct application of the test solutions to the hyphae; subsequently, the cell contents started to flow out, leading to the disruption of the cytoplasm remaining in the hyphae (Fig. 5, D and H). Occasionally, lysis did not occur only at the hyphal tips but also near septa (Fig. 5H). In separate experiments performed with *F. solani* f.sp. *phaseoli*, it was found that these were the two regions which stained with fluorescence-labeled WGA[3] indicating that chitin was freely accessible at these locations (data not shown).

DISCUSSION

Hitherto, the hypothesis of an antifungal activity of plant chitinases and β-1,3-glucanases has rested on indirect arguments (8, 9, 23). We now have demonstrated directly that chitinase and β-1,3-glucanase strongly inhibit fungal growth. It is particularly interesting that the two enzymes individually do not affect growth of most fungi. They need to be combined to exert their antifungal activity. This strongly indicates that the antifungal effect is due to the enzyme activities of chitinase and β-1,3-glucanase; it seems highly improbable that each of the two proteins cause nonspecific side effects that must be combined to inhibit fungal growth. The finding also provides a biological explanation for the coordinated regulation of chitinase and β-1,3-glucanase in plants (1, 19, 20, 24).

In combination, chitinase and β-1,3-glucanase at concentrations as low as 10 to 30 µg ml^{-1} were sufficient to inhibit growth of many phytopathogenic fungi. These inhibitory enzyme concentrations correspond to one-fourth or less of the amount present in infected pea pods, indicating that inhibition may well occur under physiological conditions. Considering that the induction of the two enzyme activities is a local reaction (data not shown), the actual enzyme concentrations in the vicinity of an invading fungus may reach inhibitory levels even earlier than indicated by the activities of the crude protein extracts.

Some plant lectins have also been reported to inhibit fungal growth; however, the concentrations required for inhibition were in the range of 1 mg mL^{-1}, *i.e.* two orders of magnitude higher (3, 10, 21). As discussed previously, some of the inhibitory effects of lectin preparations may have been due to contamination with antifungal hydrolases (26). Recently, two antifungal proteins were isolated from barley seeds (25): one, a ribosome-inactivating protein, inhibited growth of the test fungus *Trichoderma reesei* at a minimal concentration of 120 µg mL^{-1}; the second, which was recently identified as an endochitinase (17), was inhibitory at a minimal concentration of 4 µg mL^{-1}. We have observed that bean chitinase (26) and pea citinase inhibit the growth of a related fungus, *Trichoderma viride*, at similarly low concentrations.

Microscopic observations have shown that growth inhibition by the antifungal hydrolases is due to swelling and lysis of hyphal

[3] Abbreviations: WGA, wheat germ agglutinin.

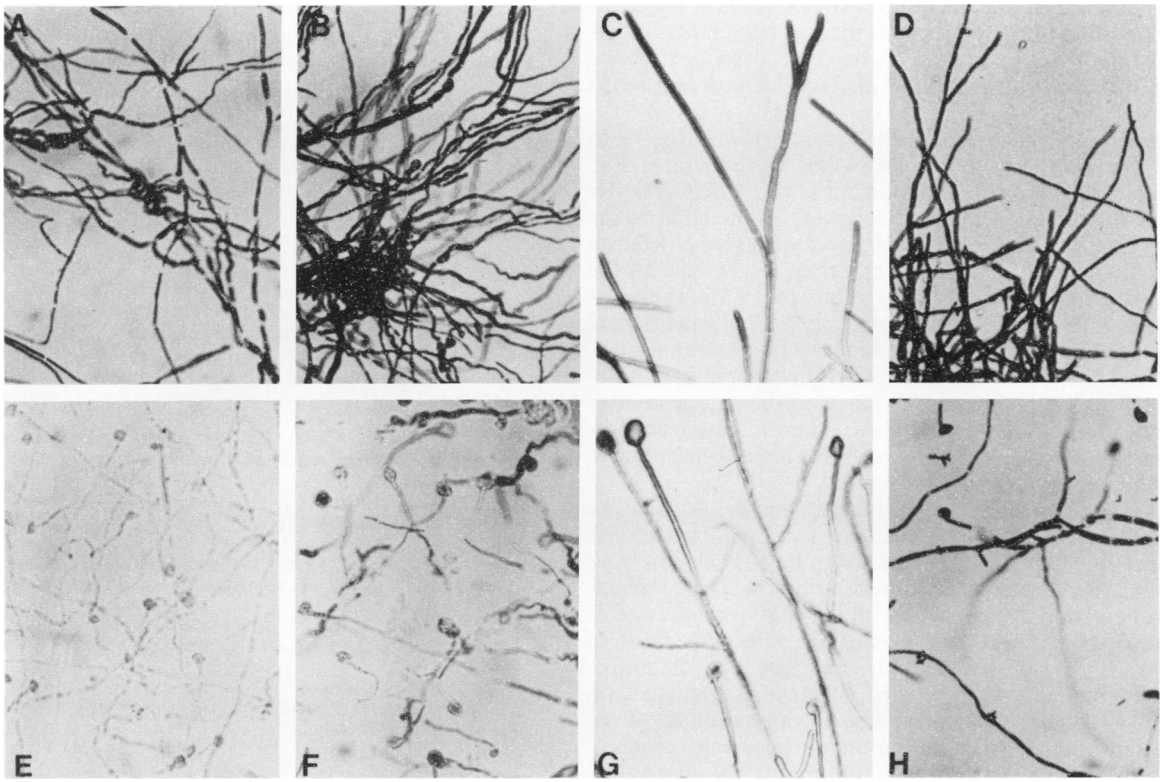

FIG. 5. Light micrographs of hyphae after incubation with crude protein extracts or purified enzymes. A, E, *F. solani* f.sp. *lycopersici* (250×), 4 h after the application of an extract (1×) from untreated pods (A) and from infected pods (E). B, F, *P. digitatum* (250×) 4 h after the application of an extract (1×) from untreated pods (B) and from infected pods (F). C, G, *T. viride* (350×) 1h after the application of water (C) and of a solution of 32 μg mL^{-1} purified chitinase Ch1 (G). D, H, *F. solani* f.sp. *phaseoli* (250×), 0 min (D) and 5 min (H) after the application of a combination of chitinase Ch1 (32 μg mL^{-1}) and β-1,3-glucanase G2 (65 μg mL^{-1}).

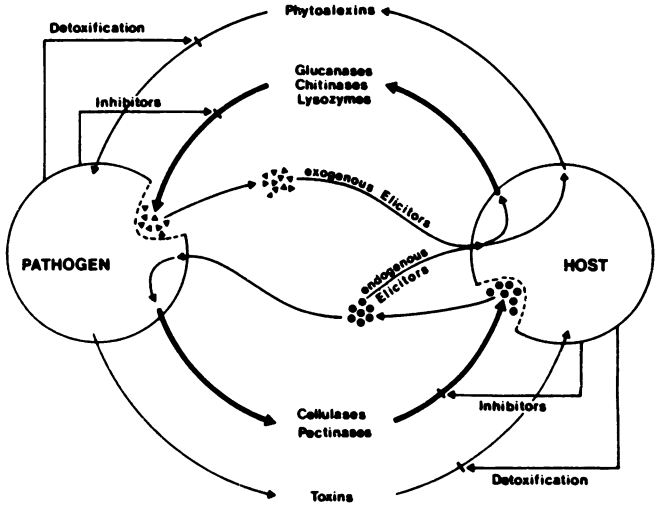

FIG. 6. Model of the action of hydrolases in plant-pathogen interactions.

tips. This is in accord with current models of hyphal growth. In fungi, wall extension is restricted to the hyphal tip and is thought to represent a delicate balance between synthesis and degradation of the main wall components, chitin and β-1,3-glucan (4, 12). Exogenously applied chitinase and β-1,3-glucanase may disturb this balance. In this regard, it is interesting that purified plant chitinase is most active on chitin in the process of formation, the so-called nascent chitin (22). Similarly, the freshly synthesized chitin at the hyphal apex is in a nascent state and, therefore particularly susceptible to chitinase. The requirement for β-1,3-glucanase in addition to chitinase to cause lysis indicates that most hyphal tips contain β-1,3-glucan in addition to chitin. Hyphal walls of subapical regions and the walls of the fungal spores were resistant to the hydrolases, suggesting that chitin and β-1,3-glucan are protected by additional compounds at these locations.

Our results do not provide any clues as to the nature of the specific factors that determine resistance or susceptibility. The pea pathogen, *Fusarium solani* f. sp. *pisi*, and the nonpathogen of peas, *Fusarium solani* f.sp. *phaseoli*, which both induce chitinase and β-1,3-glucanase to a similar extent in pea pods, are equally sensitive to the two enzymes. This clearly suggests that the differential pathogenicity of these two fungal strains is not due to differential sensitivity to pea chitinase and β-1,3-glucanase.

How is it possible that a pea pathogen, *F. solani* f.sp. *pisi*, which is apparently highly sensitive to the antifungal hydrolases on agar plates, can nevertheless invade a pea plant? One obvious possibility is that the attacking pathogen does not come into contact with the enzymes. In bean leaves, ethylene-induced chitinase (7) and β-1,3-glucanase (F. Mauch, L.A. Staehelin, in preparation) accumulate in the central vacuole. Participation of these vacuolar enzymes in defense against a fungus in the extracellular space would require the disruption of the tonoplast and the plasma membrane. This occurs generally in the hypersensitive response of plants to incompatible pathogens, a phenomenon characterized by the rapid death of a small number of cells around the infection sites. It is interesting that, in the pea-*Fusarium* interaction, only *F. solani* f.sp. *phaseoli*, but not *F. solani* f.sp. *pisi*, induced a hypersensitive reaction which became

visible about 5 h after inoculation (data not shown). An alternative possibility is that, in the plant, the pathogen forms inhibitors against the plant hydrolases, as has been described for the interaction between *Colletotrichum lindemuthianum* and beans (2).

Our results highlight a remarkable symmetry with regard to the deployment of hydrolases in host-pathogen interactions (Fig. 6). After the initial contact, each member induces the formation of hydrolases that can degrade the cell walls of the counterpart and thereby lyse its cells. Each member may also have defenses against the hydrolases of the counterpart, as in the case of the plant pectinase inhibitors (14) and in the case of the fungal β-1,3-glucanase inhibitor (2). This symmetry at the protein level complements a similar symmetry at the level of micromolecules, where pathogens form toxins and plants phytoalexins. In addition, plants may use breakdown products of the pathogen or of the host cell wall as elicitors to induce other defense reactions. Both chitinase (16) and β-1,3-glucanase (15) have been shown to release elicitors from fungal cell walls.

In conclusion, a combination of chitinase and β-1,3-glucanase strongly inhibits fungal growth. Thus, the two hydrolases clearly have an antifungal potential. It remains to be seen whether this potential actually contributes to resistance in specific plant-pathogen-interactions. Our results with *F. solani* f.sp. *pisi* and *F. solani* f.sp. *phaseoli* make it clear that the rate of increase of chitinase and β-1,3-glucanase activities does not determine whether these interactions are compatible or incompatible. Chitinase and β-1,3-glucanase are obviously not products of so-called resistance genes which determine the specificity of plant-pathogen-interactions. Rather, they are among the defense-related gene products that are induced in response to a pathogen and may contribute to the inhibition of a potential pathogen when the plant expresses resistance.

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