Prevention of the Hypersensitive Reaction in Tobacco by Proteinaceous Constituents of Pseudomonas solanacearum

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ABSTRACT

The hypersensitive reaction (HR) induced in tobacco leaves by the introduction of many incompatible bacteria can be delayed or prevented by prior infiltration with heat-killed cells of *Pseudomonas solanacearum*. A protective response, similar to that obtained with heat-killed cells, was induced by both soluble and insoluble (cell wall) fractions from disrupted bacteria. Purification of cell wall polysaccharides removed the protective properties from the insoluble fraction. The active component of the soluble fraction was precipitated with ethanol, $(NH_4)_2SO_4$, or trichloroacetic acid. Addition of four volumes of ethanol gave the most active precipitate. When resuspended in citrate buffer and infiltrated in tobacco leaves, substances in the ethanol fraction induced a protective effect within 7 hr after infiltration. The protective effect moved to nontreated leaf areas when sufficient time (48 hr) was allowed before leaves were challenged with live cells.

The active component of the ethanol fraction was destroyed by pronase, trypsin, and hot trichloroacetic acid, but was remarkably resistant to high temperatures, being completely destroyed only after prolonged autoclaving. Partial purification of proteinaceous constituents by chromatography on DEAE-Sephadex and Sephadex G-200 yielded an active fraction associated with a major protein peak. Separation of this fraction by gel electrofocusing on carrier ampholytes yielded several protein bands. Although partial protection against the HR was associated with one of the major bands, the results were not consistent, presumably due to toxicity of the carrier.

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A hypersensitive reaction (HR) is induced in plants after introduction of many incompatible, pathogenic bacteria (8). In leaves, the reaction is characterized in its initial stages by rapid loss of electrolytes (1, 3) and damage to membranes of cellular organelles (4). In tobacco leaves, cellular collapse, bleaching, and desiccation in the affected areas are usually complete by 18 hr after infiltration of the intercellular spaces with 10^8 cells/ml of a wide variety of incompatible bacteria. The HR induced in tobacco leaves by bacteria may be prevented by various means, such as (i) exposure of infiltrated leaves to 36 C (8), or to darkness (14); (ii) application of a streptomycin solution within 20 min after leaves are infiltrated with live bacteria (9); (iii) the use of calcium nitrate in the inoculum suspension (2); and (iv) prior infiltration with suspensions of heat-killed bacteria (15).

The use of heat-killed bacteria is of particular interest, as the protective response obtained is both time and light dependent, and becomes systemic. Given sufficient time and under suitable light conditions, it is possible to protect leaves located above and below an infiltrated leaf. In the protected areas, numbers of live bacteria drop precipitously soon after infiltration, although there are no outward symptoms of a host response to the pathogen (15).

The protective effect of heat-killed bacteria is not specific, and is equally effective against compatible (11) and incompatible (15) bacteria, as well as against local lesion formation by tobacco mosaic virus (10). Because the HR caused by incompatible bacteria can be adequately controlled and is highly reproducible, the reaction has been used to determine the protective effectiveness of various species of bacteria as well as of cell-free fractions prepared from these bacteria (17, 18). These initial studies suggest that the protective factor from heat-killed cells of *Pseudomonas solanacearum* is proteinaceous. The purpose of the present paper is to report additional evidence which confirms this initial conclusion, and to describe some of the properties of the purified protection factor.

MATERIALS AND METHODS.—*Extraction of the protection inducer.*—Two isolates of *P. solanacearum*, B-1, a cultural mutant of isolate K-60 (isolated from tomato, *Lycopersicon esculentum* Mill.), and S-210 (isolated from plaintain, *Musa* group AAB) were used in most experiments. Both isolates induced a rapid HR when infiltrated on tobacco leaves (14), but B-1 was preferentially used because it does not produce extracellular polysaccharides (7) which interfere in the fractionation of bacterial proteins. The isolates were streaked on a tetrazolium medium (7) and incubated for 48 hr at 32 C. Individual colonies were used as a source of inoculum for batch cultures which were grown in 2.8-liter Fernbach flasks containing 500 ml of the following medium: 5 g Bacto tryptone, 5 g dextrose, 0.5 g yeast extract, and 1 liter distilled water. Culture flasks were placed on a rotary shaker and incubated at 28 C for 48 hr.

Bacteria were collected by centrifugation at 6,500 *g* for 20 min, and were then suspended in 0.02 M citrate buffer (Na^+), pH 6.0, containing 1 g/liter ascorbic acid. Approximately 20 ml of buffer/5 g of bacteria (fresh wt) were used. The cells were ground for 3 min at high speed with small glass beads in a Bronwill MSK cell disintegrator cooled with CO_2. All subsequent fractionation procedures were carried out

in the cold (4 C) unless otherwise indicated. The broken cells were removed by centrifuging twice at 17,000 *g* for 40 min, and the supernatant fluid was filtered through a Millipore filter (0.45 μ) and dialyzed overnight against several volumes of citrate buffer. Four volumes of 95% ethanol were added to the remaining fluid, and the mixture was allowed to stand overnight. In some experiments, dialysis was omitted and the ethanol was added directly to the supernatant fluid.

A flocculent, white precipitate was recovered after centrifuging at 8,000 *g* for 20 min. The precipitate was suspended in one-fifth to one-eighth of the original volume of buffer, and the suspension centrifuged at 17,000 *g* for 30 min. The volume of the clear supernatant was reduced to one-half by vacuum evaporation at 35 C or by placing the liquid in a dialysis bag in contact with dry Aquacide II (Fisher Scientific Co., Chicago, Ill.). The latter method was preferred because it was more rapid than evaporation and presented no foaming problems. The clear solution obtained (referred to as ethanol fraction) contained a mixture of nucleic acids, cell wall polysaccharides, cellular organelles, and soluble proteins.

Fractionation of the ethanol fraction was carried out on a Sephadex G-200 column (2.5 X 38.0 cm) equilibrated with either 0.02 M citrate buffer (Na^+), pH 6.0, or 0.02 M phosphate buffer (Na^+), pH 6.5. A solution of blue dextran was layered on top of the column prior to each run in order to check column performance. A sample of ethanol fraction in buffer, containing 10-60 mg protein, was layered when the blue dextran had moved approximately 1.0 cm into the bed. Thirty 4-ml fractions, containing most (95%) of the protein in the original sample, were collected and assayed immediately for protective activity or stored at 10 C until assayed. Protein was determined by the method of Lowry et al. (13).

Additional purification of the active component was attempted by chromatography on DEAE-Sephadex and by acrylamide gel electrofocusing. Active fractions from Sephadex G-200 columns absorbed strongly at 260 nm, indicating contamination with nucleic acids. Pooled fractions were dialyzed overnight in several 1-liter volumes of 0.05 M Tris [tris (hydroxymethyl) amino methane] buffer, pH 7.5, and were applied to a 2- X 20-cm column of DEAE-Sephadex. Proteins were separated by a stepwise NaCl gradient in Tris buffer until 0.4 M was reached. A large protein peak was obtained by elution with 0.26 - 0.30 M NaCl. These fractions were pooled and concentrated by addition of Aquacide II. The concentrate exhibited a single peak at 280 nm, and was assayed for protection against the HR in the usual manner. Substances in this concentrate gave full protection against the HR within 24 hr after infiltration. This concentrate, or similar concentrates from fractions eluted from Sephadex G-200 columns, were separated by electrofocusing in acrylamide gels containing pH 3 to 10, 7 to 9, or 6 to 8 ampholine carriers. Approximately 0.3 mg protein was applied to each gel tube. Constant current (1.5 ma/tube) was applied for 4 hr.

Assay.—Heat-killed cells (15), ethanol fraction, and purified column fractions were assayed for their capacity to prevent the HR in tobacco leaves. Seedlings of *Nicotiana tabacum* L. 'Bottom Special', grown in vermiculite for ca. 30 days, were transplanted one to a 6-inch pot containing coarse silica sand. Plants were watered daily with Hoagland's nutrient solution and grown in either (i) a growth room at 28 C, 50% relative humidity, and 1,200 ft-c on a 12-hr photoperiod provided by Sylvania 'Gro-Lux' and General Electric cool-white fluorescent tubes and tungsten incandescent bulbs; or (ii) the greenhouse at 28 C (±8 C).

The sixth and seventh fully expanded leaves numbered from the base of the plant were used for the assay. Solutions were injected into the intercellular spaces of alternate intercostal leaf areas (panels) with a hypodermic syringe fitted with a fine needle (No. 30). When the panels appeared water-soaked, infiltration was judged to be complete. The small panels located near the base and tip of the leaf laminae were not used in most tests because their response was variable. After 24 hr, one or two small areas (ca. 2 cm^2 each) in the center of each panel were infiltrated with a suspension of 10^8 cells of either B-1 or S-210 strains of *P. solanacearum*. In some instances, a separate small area in each panel was infiltrated 24 hr after the first challenge. A typical HR was obtained in untreated control panels, or in those previously injected only with distilled water or buffer. In those panels previously injected with heat-killed cells, ethanol fraction, or column fractions, the challenge inoculation resulted 24 hr later in the following: (i) complete protection, i.e., no evident reaction (index 4); (ii) intermediate protection, in which three-fourths (index 3), one-half (index 2), or one-fourth (index 1) of the challenged area remained green while the remainder of the tissue collapsed; and (iii) a normal HR (index 0) in which the tissue in the entire treated area collapsed.

RESULTS.—*Biological activity and other properties of crude cell-free extracts.*—Disintegration of bacterial cells by glass beads yielded a suspension which was separated by centrifugation into an insoluble fraction, consisting mostly of cell wall material, and a "soluble" (clear) fraction. Infiltration of both fractions into tobacco leaves resulted in complete prevention of the HR within 24 hr in the treated panels; and within 48-60 hr, in the adjoining, untreated panels. Because the insoluble fraction appeared to be considerably more effective (as determined by dilution end points) than the soluble components, attempts were made to determine if cell wall polysaccharides were associated with the protective response. Cell wall polysaccharides were purified by the procedure of Hancock & Park (5), which involves extraction of bacterial cells with 5% trichloroacetic acid at 100 C for 15 min, digestion of the residue at 37 C with trypsin (500 μg/ml) in 0.01 M ammonium bicarbonate (pH 8.0), and the washing of the residue with ammonium bicarbonate

and 1 N ammonia. This fraction consisted almost entirely of cell wall material, but was completely ineffective in preventing the HR in tobacco leaves.

A protective effect was obtained, on the standard assay, by *P. solanacearum* cells which had been disrupted by a 5-sec treatment with ultrasonic energy from a Bronson probe sonifier, Model LS-75. Protracted sonication (over 1 min), however, caused considerable loss of activity, even when provisions were made to cool the suspension during sonication.

The results suggested that the protection factor could involve mucopeptides, proteins, or other labile constituents of bacterial cell walls, which were degraded by the procedures outlined above. Initial studies indicated that most of the protective activity of the soluble fraction from disrupted cells (hereafter referred to as "crude extract") could be removed by addition of trichloroacetic acid (5%, w/v), $(NH_4)_2 SO_4$ (35%, w/v), or four volumes of ethanol (95%, v/v). The precipitate obtained by any of these treatments, when suspended in citrate buffer, had very high protective activity. A comparison of the protective activities of the ethanol precipitate and of the crude extract indicated that, on the basis of protein content, the crude extract was the more active (Table 1). Evidently, not all the protection factor is precipitated by ethanol or, alternatively, a portion of this factor is inactivated by the treatment. In general, the crude extract induced complete protection at 0.1 mg protein/ml or higher, but protection was variable below this concentration. At low protein concentrations, protection usually was not complete; i.e., the challenge inoculation resulted in variable amounts of collapsed tissue within the infiltrated area. In other instances, however, protection appeared complete after 24 hr, but a delayed HR developed after 48 hr.

Trichloroacetic acid (TCA) and $(NH_4)_2 SO_4$ were not so effective as ethanol, at the concentrations used, for precipitation of the protective factor from crude preparations. When prepared from equivalent volumes of crude extract, ethanol precipitates were invariably more effective, as indicated by dilution end points, than the other two preparations. Precipitation with TCA (Table 2) was less effective than ethanol precipitation, presumably owing to protein denaturation. Treatment of a cold TCA precipitate with hot (78 C) TCA for 6 min resulted in further reduction or complete loss of biological activity.

Suspensions of the crude extract in phosphate buffer, at a concentration of 0.1 mg protein/ml, induced no protection if leaf areas were challenged with live cells immediately after infiltration. Full protection was obtained after 7, 19, and 48 hr, however. No effort was made to determine if protection was maintained beyond 48 hr (Table 3).

Some of the variability encountered in the degree of protection obtained with each individual preparation may have been due to differences in age of the test leaves or in the location of the area infiltrated on each leaf. Comparisons of the protection induced by crude extracts in infiltrated panels located at the tip, middle, and basal portions of the sixth and seventh leaves indicated that at high protein concentrations, protection was consistently

TABLE 1. Effect of crude and ethanol fractions of *Pseudomonas solanacearum* on the development of the hypersensitive reaction (HR) in tobacco leaves

Protein concn. mg/ml	HR index[a] on leaves 48 hr after challenge with live B-1 cells (10^8/ml)	
	Crude	Ethanol fraction
0.200	4.0	3.7
0.100	4.0	3.3
0.050	3.5	2.6
0.025	3.2	0.5
0.012	2.0	0.8

[a]HR index ranged from 0.0 = normal HR, i.e., no protection; to 4.0 = no reaction; i.e., complete protection; see text for details. Averages of four determinations each on fifth and sixth leaves from the base.

TABLE 2. Effect of trichloroacetic acid precipitate (1.0 mg protein/ml) of *Pseudomonas solanacearum* on prevention of the hypersensitive reaction (HR) in tobacco leaves

Dilution	HR index[a] on leaves challenged after:			
	24 hr		48 hr	
	Cold TCA[b]	Hot TCA[c]	Cold TCA	Hot TCA
Full strength	4.0	3.0	2.0	1.0
1:10	4.0	3.0	0.0	0.0
1:50	2.0	0.0	0.0	0.0
1:100	2.0	1.0	0.0	0.0

[a]HR index as in Table 1. Determined 48 hr after challenge with live B-1 cells (10^8/ml). Each value represents the average of four determinations.

[b]TCA (5%) added to the crude extract at 4 C and the ethanol precipitate (ppt), then washed with 95% ethanol and suspended in citrate buffer (0.02 M, Na^+).

[c]Cold TCA ppt treated with hot (78 C) TCA (5%) for 6 min, washed with 95% ethanol, and suspended in citrate buffer.

TABLE 3. Effect of crude extract (0.1 mg protein/ml) of *Pseudomonas solanacearum* on prevention of the hypersensitive reaction (HR) in relation to time allowed before challenging with live cells

Leaves challenged after (hr)	HR index[a] on:	
	Leaf 1	Leaf 2
0	0.0	0.0
2	0.5	2.3
7	3.8	3.9
19	3.8	3.8
48	3.7	3.5
LSD_{05}:0.3		
LSD_{01}:0.5		

[a]HR index as in Table 1. Determined 48 hr after challenge with live B-1 cells (10^8/ml). Each value represents the average of four-six determinations on two leaves from three plants.

TABLE 4. Effectiveness of a crude extract of *Pseudomonas solanacearum* in preventing the hypersensitive reaction (HR) at different locations in tobacco leaves

	HR index[a] at:			
	0.2 mg protein/ml		0.02 mg protein/ml	
Location on leaf	Leaf 6	Leaf 7	Leaf 6	Leaf 7
Base	2.7	3.8	1.6	1.0
Middle	3.5	3.8	3.7	2.2
Tip	3.7	3.9	2.7	0.9
LSD_{05}:0.2				
LSD_{01}:0.4				

[a]HR index as in Table 1. Determined 48 hr after challenge. Each value represents the average of nine determinations on sixth or seventh leaves (from the base) from three plants.

[b]Entire leaf panel at the base, middle, or tip of the leaf was infiltrated with crude extract, and each panel was challenged 24 hr later with live cells of strain B-1 (10^8/ml).

more effective at the middle or tip of the leaf than at the base. At low protein concentrations, protection was highly variable, particularly at the tip and basal ends of the leaf (Table 4).

It seemed important to determine if the medium used to grow *P. solanacearum* contained materials which could induce protection. Ethanol precipitate obtained from noninoculated standard medium (2 liters maintained under constant agitation for 48 hr) did not induce protection when suspended in citrate buffer and infiltrated in tobacco leaves. Cells of *P. solanacearum* grown in Husain-Kelman medium (6) for 48 hr yielded, after extraction by the standard procedure, a protective factor which had identical properties to that obtained from cells grown in the standard medium. Small amounts of a similar substance could be precipitated by addition of 4 volumes of ethanol to the culture medium (2 liters) after bacterial cells were removed by filtration through a Millipore filter.

The possibility that protection could be the result of a generalized response of leaf tissues to proteins, polysaccharides, nucleic acids, or other polymers which precipitate after addition of ethanol to crude extracts was examined. The extracellular polysaccharide of isolate K-60 was purified by the method of Husain & Kelman (6). The polysaccharide extracted from 2 liters of culture medium was dissolved in 25 ml of distilled water and assayed for protective activity in the usual manner. The HR was not prevented on leaves infiltrated with this solution, although the concentration of polysaccharide was sufficient to cause temporary flaccidity of infiltrated tissues.

Purified DNA from *Pseudomonas solanacearum* (courtesy of David Coplin), as well as calf thymus DNA and *Escherichia coli* RNA (Nutritional Biochemicals Corp., Cleveland, Ohio), each at 1 mg/ml, failed to induce a protective response on tobacco leaves. Since the protection factor did not involve cell wall or extracellular polysaccharides or nucleic acids, it seemed likely that proteins with unusual heat stability could be the active agents. The protective properties of the ethanol fraction were reduced or eliminated after treatment with proteolytic enzymes such as trypsin or pronase. Ethanol fraction was suspended in phosphate buffer (K^+), pH 7.0, and 1 ml was mixed with 0.5 ml trypsin solution (1 mg/ml). The mixture was incubated at 34 C for 2.5 hr, then placed for 10 min in a boiling water bath to stop the reaction. Alternatively, the enzyme was removed by eluting the mixture through a short column of Sephadex G-50, which retards trypsin but allows the protective factor to move through readily. In all cases, trypsin reduced or completely destroyed the protective properties of the ethanol fraction or of other biologically active fractions (Table 5). Similar results were obtained when ethanol fraction was treated with pronase. The preceding experiments indicated that the protective factor was a protein, or a glycoprotein, with specific properties. No protection, for example, was obtained by infiltration of tobacco leaves with casein (1% and 2%, w/v), trypsin (0.5 g/ml), horseradish peroxidase (50 and 100 mg/ml), or ribonuclease (0.5 mg/ml).

Chromatography of the ethanol fraction.—That the protection factor is a protein of large size and of unusual properties was confirmed after separation of the ethanol fraction by chromatography on Sephadex columns. The protein was not retarded by Sephadex G-50, and did not separate properly from other proteins when eluted through Sephadex G-100. Best separation was obtained with Sephadex G-200, which has an exclusion limit of 200,000 mol wt. Protection was associated with a broad peak of protein eluting at 56-76 ml beyond void volume (fractions 14-19, Fig. 1); peaks of protein and protection were nearly coincident. The protective properties of column

TABLE 5. Effect of trypsin digestion on the capacity of the ethanol precipitate fraction of *Pseudomonas solanacearum*, and of a similar fraction eluted through a Sephadex G-200 column, to prevent the hypersensitive reaction (HR) in tobacco leaves

	HR index[a] in panels infiltrated with:			
Substrates	Trypsin + substrate[b]	Trypsin + buffer	Heat-inactivated trypsin + substrate	Buffer + substrate
Ethanol ppt (1.2 mg protein/ml)	2.0	0.0	4.0	4.0
Column fractions[c] (0.4 mg protein/ml)	0.0	0:0	4.0	4.0

[a]HR index as in Table 1. Determined 48 hr after challenge with live B-1 cells (10^8/ml). Each value represents the average of two determinations.

[b]Incubation mixture: 1 ml substrate and 0.5 ml trypsin (1 mg/ml) incubated at 34 C for 2.5 hr.

[c]Pooled fractions corresponding to 56-76 ml in Fig. 1.

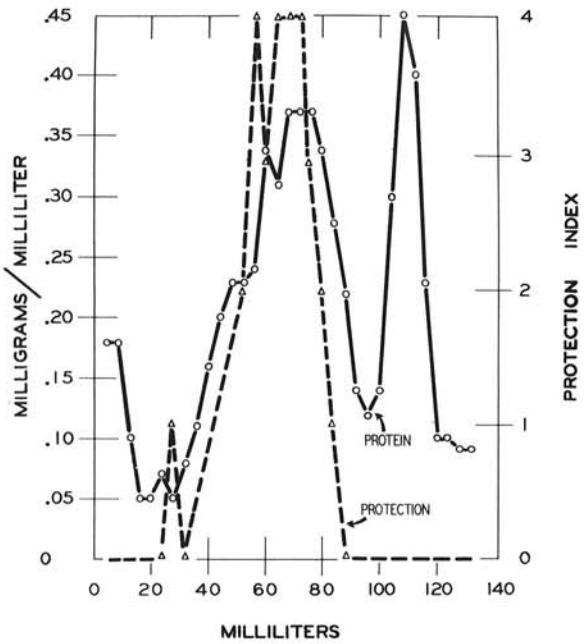

Fig. 1. Fractionation of the ethanol fraction of *Pseudomonas solanacearum* on Sephadex G-200. A sample containing 30 mg protein was eluted with 0.02 M phosphate buffer (Na^+) and each 4-ml fraction was assayed for protection against the hypersensitive reaction on tobacco leaves and for protein content.

fractions 14-19 were destroyed by trypsin under the same conditions previously described for the ethanol fraction (Table 5).

The compounds in biologically active column fractions showed remarkable heat stability. In one experiment, pooled column fractions were heated for 10 min in a boiling water bath and, after centrifugation at 8,000 *g* for 30 min, the supernatant fluid was assayed for biological activity. Heat treatment of this intensity reduced, but did not completely destroy, the protective properties of substances in these fractions (Table 6). Untreated control fractions could be diluted 1:200 without loss of biological activity, but treated ones gave complete protection only at full strength. Other experiments have shown that protracted (1 hr) autoclaving at 15 lb. pressure will completely destroy the protection activity of crude cell-free preparations, however.

TABLE 6. Effect of heating on the capacity of Sephadex G-200 column fractions from the ethanol fraction of *Pseudomonas solanacearum* to prevent the hypersensitive reaction (HR) in tobacco leaves

Treatment	HR index[a] in panels infiltrated with column fractions[b] at dilutions of:			
	1	1:10	1:100	1:200
Control (unheated)	4.0	4.0	4.0	4.0
Heated (95 C for 10 min)	4.0	2.0	2.0	0.0

[a]HR index as in Table 1. Determined 48 hr after challenge with live B-1 cells (10^8/ml). Each value represents the average of three determinations.

[b]Pooled fractions, corresponding to 56-76 ml in Fig. 1. Protein concentration ca. 2.4 mg/ml.

Concentrates from DEAE column fractions separated by isoelectric focusing on pH 6-8 gels showed one major band, with an isoelectric point of ca. pH 6.8, and other minor bands crowded at the acidic end of the gel. Separations on similar gels of concentrated fractions from Sephadex G-200 columns showed several bands, the major one having an isoelectric point at ca. pH 7.2.

In order to determine the biological activity of different protein bands, attempts were made to elute them from the acrylamide gels. Difficulties were experienced in eluting the proteins and in removing the toxic ampholine carriers. Suspensions prepared by grinding gel slices in 0.02 M citrate buffer (Na^+), pH 6.0, gave toxic reactions on tobacco leaves and protection against the HR was erratic. Partial protection was associated with the major protein band, but the results were not consistent, presumably due to toxicity of the carrier. Attempts to separate the proteins from the carrier (16) have not been successful.

DISCUSSION.—Our results indicate that the ability of heat-killed cells of *Pseudomonas solanacearum* to induce a protective reaction in tobacco leaves (15) is due to bacterial proteins or proteinaceous complexes which have outstanding heat stability. It is not possible at present to conclude whether one or several proteins are involved, because the assay of individual protein bands separated by isoelectric focusing on acrylamide gels proved difficult. Nevertheless, induction of protection appeared associated with one major band. Whether other proteins have this property is unknown. An answer to this question depends on the development of appropriate techniques for isolation of sufficient amounts of this and other proteins from active fractions. The biologically active substance may be a glycoprotein, and is most likely associated with the bacterial cell wall.

The catalytic properties of two well-known heat-resistant enzymes, ribonuclease and peroxidase, were destroyed by exposure to heat treatments that did not markedly affect the biological activity of the protection factor. Also, these enzymes, unheated, did not induce a protective effect when infiltrated at high concentrations.

The results of chromatography on Sephadex columns indicate that the protection factor has a high molecular weight, probably as high as 150×10^3. It is only partly solubilized by the methods used to disrupt bacterial cells; much of the biological activity remained associated with wall materials even after bacterial cells were broken up by high-speed grinding with glass beads. Although extraction of this factor from bacteria other than *P. solanacearum* has not been attempted, it is likely that it exists in other species of *Pseudomonas* and *Xanthomonas* which induce protection against the HR when heat-killed cells are infiltrated in tobacco leaves (15).

It is apparent that heat treatment is not necessary

for the release or activation of the protection factor from *P. solanacearum*, as was suggested previously by Lozano & Sequeira (15). Sonication or grinding with glass beads releases large amounts of the protection protein from bacterial cells, and small amounts are also released into the culture medium during growth of the organism, presumably as a result of autolysis. One can speculate that the bacterium releases small amounts of these proteins within the host tissues, but not in amounts sufficient to induce an effective protective response. In tobacco leaves, incompatible bacteria, such as isolate B-1, presumably release both the HR-inducing factor and the factor that prevents the HR, but the response to the first is so rapid that the effect of the second is probably masked. Both factors appear to be proteinaceous, and are produced by a wide variety of plant-pathogenic bacteria (17, 18).

The proteins from *P. solanacearum* capable of preventing the HR are probably similar to those extracted from *P. tabaci* (12), and capable of inducing resistance to infection by the wildfire bacterium. Because peroxidase activity increased in tobacco leaves after injection with these extracts, or with heat-killed cells of the same bacterium, it was suggested that peroxidase was the substance responsible for the protective effect. Although infiltration with heat-killed cells of *P. solanacearum* causes a substantial increase in peroxidase activity of young tobacco leaf cells, we did not obtain a delay in appearance of the HR after direct injection of peroxidase at concentrations reported to protect against infection by the wildfire bacterium (12).

The lack of specificity of the protective response of tobacco leaves induced by bacterial proteins is of particular interest. In addition to their effect on the HR, preparations from *P. solanacearum* reduce both the number and size of lesions induced by local lesion strains of TMV on tobacco leaves (*unpublished data*). Similar results have been obtained with heat-killed cells of *P. syringae* by Loebenstein & Lovrekovich (10), who reported that this protective effect was inhibited by actinomycin-D, suggesting that RNA synthesis is required. The precipitous drop in bacterial numbers after infiltration of protected panels, and the reduction of viral multiplication, suggest rapid induction of a general mechanism for disease resistance in plants.

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