Host-Pathogen Interactions

XIV. ISOLATION AND PARTIAL CHARACTERIZATION OF AN ELICITOR FROM YEAST EXTRACT[1]

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ABSTRACT

An elicitor of glyceollin accumulation in soybeans (*Glycine max* L.) has been isolated from a commercially available extract of brewers' yeast. Yeast is not a known pathogen of plants. The elicitor was isolated by precipitation in 80% (v/v) ethanol followed by column chromatography on DEAE-cellulose, sulfopropyl-Sephadex, and concanavalin A-Sepharose. Compositional and structural analysis showed the elicitor to be a glucan containing terminal, 3-, 6-, and 3,6-linked glucosyl residues. The yeast elicitor stimulates the accumulation of glyceollin in the cotyledons and hypocotyls of soybeans when as little as 15 nanograms or 100 nanograms of the elicitor is applied to the respective tissues. The yeast elicitor is very similar in both structure and absolute elicitor activity to an elicitor isolated from the mycelial walls of *Phytophthora megasperma* var. *sojae*, a pathogen of soybeans. These and other results of this laboratory suggest that plants are able to respond to the presence of a wide range of fungi by recognizing, as foreign to the plant, structural polysaccharides of the mycelial walls of the fungi.

Plants accumulate toxins, phytoalexins, in response to invading pathogens. The accumulation of phytoalexins is thought to play a major role in disease resistance and occurs in response to a wide variety of microorganisms, both pathogenic and nonpathogenic (13, 17, 22, 23). Phytoalexins have been shown to have a broad spectrum of antimicrobial activity, affecting not only some pathogenic microorganisms, but many nonpathogenic ones as well (2, 3, 17). Cell-free filtrates from cultures of a number of different microorganisms have been shown to induce the production of phytoalexins (4, 5, and refs. therein). Molecules which elicit phytoalexin accumulation have been isolated from the mycelial walls of two species of *Phytophthora* (6, 7, 10), as well as from *Colletotrichum lindemuthianum* (4). Such molecules which stimulate phytoalexin accumulation are called elicitors. Soybean tissues are able to respond to as little as 10⁻¹² moles of the *Phytophthora megasperma* var. *sojae* elicitor (5).

The mycelial elicitors are glucans (4, 7). Ayers *et al.* (6, 7) have demonstrated that the glucans isolated from each of three races of *P. megasperma* var. *sojae* have the same glucosyl linkage compositions and possess identical abilities to stimulate the accumulation of phytoalexins in soybean tissue. In contrast to an earlier report (20), Ayers *et al.* (7) found that the *P. megasperma* var. *sojae* elicitor cannot account for the varietal specificity exhibited by the *Phytophthora* races for their host, soybean. Ayers *et al.* (7) suggested that the elicitor response was a part of a general defense mechanism of plants, a mechanism which might be activated by

a wide variety of microorganisms. If this is indeed the case, then one should be able to find elicitors in the mycelial walls of other fungi. The results reported in this paper demonstrate that an autolysate of brewers' yeast (*Saccharomyces cerevisiae*) possesses an elicitor very similar to the *Phytophthora* elicitor. These results support the hypothesis that the elicitor-stimulated accumulation of phytoalexins represents a widespread method by which plants defend themselves.

MATERIALS AND METHODS

Materials. Yeast extract, an autolysate of brewers' yeast (*Saccharomyces cerevisiae*), was obtained from Difco Co., Detroit. The glucan elicitor from *P. megasperma* var. *sojae* was obtained from B. S. Valent and had been isolated from race 1 of the fungus by procedures previously published (6). Harosoy 63 soybean seed was obtained from J. Paxton, Department of Plant Pathology, University of Illinois at Urbana-Champaign.

Assays for Elicitor Activity. The cotyledon and hypocotyl assays for elicitor activity (5) were used with some modifications. Antibiotic was not used, and the samples to be assayed were dissolved in autoclaved, deionized H₂O. Seventy-five μl of a sample to be tested for elicitor activity was applied to each cotyledon or 20 μl of a sample was applied to each hypocotyl in the respective assays. These changes did not alter the response of either cotyledons or hypocotyls to control solutions or to standard elicitor preparations isolated from *P. megasperma* var. *sojae*.

The elicitor activity (A₂₈₅) of the various fractions obtained during the isolation of the yeast extract elicitor was not the same. One way of comparing the relative elicitor activities is by determining the amount (μg of carbohydrate) of each fraction required to give an arbitrary A₂₈₅ value (6). In the present study, an arbitrary A₂₈₅ value of 0.5 was chosen as the point of comparison. The relative activities reported in this paper are all normalized to the activity of the concanavalin A-Sepharose-purified elicitor.

Analysis of the Sugar and Glycosyl Linkage Composition. Neutral sugar compositions were determined by the method of Jones and Albersheim (18). The partially methylated alditol acetate derivatives were prepared as described previously (32) with one modification. The methylated polysaccharide was hydrolyzed using 90% formic acid at 100 C for 5 hr, followed by treatment with 2 N trifluoroacetic acid at 121 C for 1 hr. The separation of the various sugar derivatives was achieved by GC on a Hewlett-Packard model 7620A gas chromatograph using glass columns containing 0.2% ethylene glycol succinate, 0.2% ethylene glycol adipate, and 0.4% XF-1150 silicone oil on Gas-chrom P (100–200 mesh) prepared as described (18).

The alditol acetates of the neutral sugars were separated by a temperature program of 2 C/min from 140 to 190 C following a postinjection interval of 2 min. The partially methylated alditol acetates were separated by a temperature program of 1 C/min from 130 to 190 C after a postinjection interval of 2 min. The upper temperature in both instances was maintained until no

further peaks were observed eluting from the column. Nitrogen, at a flow rate of 25 ml/min, was used as a carrier gas. The alditol derivatives were identified and quantitated by methods that have been described (18, 32). Since the partially methylated alditol acetate derivatives of 4- and 6-linked glucosyl residues co-chromatograph on the column described above, the samples were also injected on an OV-275 column prepared according to Darvill *et al.* (12). This column sufficiently separates the 4- and 6-linked glucosyl derivatives to permit quantitation.

Combined Use of Anthrone and Alditol Acetate Methods for Determination of Carbohydrate Content of Elicitor Fractions. The absorbance obtained in the anthrone assay (15) for equal amounts of different sugars is not uniform. The carbohydrate content of elicitor preparations has been calculated from the alditol acetate-determined sugar composition in combination with the absorbance obtained in the anthrone assays using the equation described by Ayers *et al.* (6).

RESULTS

PURIFICATION OF THE ELICITOR

Ethanol Precipitation. The steps involved in the isolation from yeast extract of an elicitor of glyceollin accumulation in soybeans are summarized in Table I. A typical isolation of the elicitor was initiated by dissolving 200 g of yeast extract in 1 liter of deionized H_2O. Ethanol was added to 80% (v/v). The precipitate was allowed to settle for 4 days at 6 C and the supernatant solution was decanted and discarded. The gummy precipitate, which remained in the flask, was dissolved in 1 liter of deionized H_2O. The ethanol precipitation was repeated. The second ethanol precipitate was dissolved in 800 ml of deionized H_2O. This preparation is henceforth referred to as the "crude" fraction.

The crude fraction was dialyzed extensively against deionized H_2O at 6 C. The precipitate which formed inside the dialysis bag was removed by filtration through a 1.2-μm Millipore filter. The neutral sugar composition of this fraction (Table I), as determined by the alditol-acetate method, indicated the presence of ribose, mannose, and glucose, as well as small amounts of arabinose and galactose. The dialyzed fraction was made 60% (v/v) with respect to ethanol. The precipitate which formed was pelleted by centrifugation at 21,500g for 15 min. The supernatant solution was decanted and evaporated under reduced pressure at 40 C to a volume of 350 ml. This solution contained 4.1 g of carbohydrate as determined by the combined anthrone-alditol acetate method. Further purification of the 60% (v/v) ethanol-soluble, 80% (v/v) ethanol-insoluble elicitor was achieved by column chromatography.

DEAE-Cellulose Chromatography. A portion (160 ml of 350 ml containing 1.86 g of carbohydrate) of the dialyzed 60% (v/v) ethanol-soluble fraction was applied to a DEAE-cellulose (Whatman) column (2.5 × 25 cm) which had been equilibrated at 6 C with 5 mM Na-phosphate (pH 7.7). The column was eluted with 60 ml of the equilibration buffer and 5-ml fractions were collected.

The bound material was eluted from the column by a linear NaCl gradient (0–0.5 M) prepared in the same buffer (total gradient volume was 250 ml). About 70% of the carbohydrate-containing material had no affinity for the DEAE-cellulose column. Compositional analysis of the elicitor-active fraction showed the presence of mannose and glucose, and small amounts of arabinose and galactose. This fraction still contained Lowry-positive (24) material (data not shown). No ribosome was detected in this fraction (Table I). The relative elicitor activity of this fraction was 6 (Table I).

Three relatively small peaks of carbohydrate-containing material eluted from the DEAE-cellulose column in the NaCl gradient. Compositional analysis showed that two of these peaks contained predominantly mannose (>90%) with only small amounts of glucose, while the third contained almost exclusively ribose. None of these fractions possessed significant elicitor activity as evidenced by their low relative elicitor activities (0.5 or less).

The elicitor-active material from the DEAE-cellulose column was concentrated to 75 ml under reduced pressure at 40 C. This sample was then dialyzed against 10 liters of deionized H_2O for 24 hr to yield 986 mg of carbohydrate in 98 ml.

Sulfopropyl-Sephadex Chromatography. The pH of the dialyzed void fraction from the DEAE-cellulose column was adjusted to 3.5 with dilute acetic acid. A portion (20 ml of 98 ml) of this solution containing 202 mg of carbohydrate was then applied to a sulfopropyl-Sephadex (Sigma) column (2.5 × 20 cm). The column, which had been equilibrated with 20 mM sodium acetate (pH 3.5) was eluted at 6 C with 200 ml of the equilibration buffer. Three-ml fractions were collected. The material which was retained by the column was eluted with a linear NaCl gradient (0–1 M) prepared in the same buffer (total gradient volume was 400 ml). The column effluent was monitored for carbohydrate by the anthrone assay and for protein by the A at 289 nm. Most of the carbohydrate-containing material (60%) was not retained by the column. Only 12% of the Lowry-positive material (24) passed through the column, the remainder coming off the column in the salt gradient. Compositional analysis of the carbohydrate in the void peak and in the peaks eluting from the column in the salt gradient showed that the void material had a higher percentage of glucose than did the fractions which remained bound to the column (11% versus 4% and 8%). All of the peaks eluting from the column had similar relative elicitor activities. However, attention was focused on the void peak since this peak contained most of the carbohydrate and the least amount of protein.

The elicitor-active fractions from the void peak of the sulfopropyl-Sephadex column were pooled and reduced to 19 ml by evaporation under reduced pressure at 40 C. This did not affect the ability of this fraction to stimulate the production of glyceollin in soybean tissue. This elicitor-active fraction contained 118.6 mg of carbohydrate.

Concanavalin A-Sepharose Chromatography. A 5-ml aliquot containing 31.2 mg of carbohydrate, of the void material from the sulfopropyl-Sephadex column was evaporated to approximately 1 ml and diluted 1:3 with concanavalin A buffer (100 mM sodium acetate [pH 5.2] containing 150 mM NaCl, 1 mM MgCl₂, 1 mM MnCl₂, and 1 mM CaCl₂) and applied to a column (1.5 × 19 cm) of concanavalin A covalently linked to Sepharose 4B (Pharmacia). The column had been washed previously with at least 1 liter of concanavalin A buffer. Due to the high mannose content of the sample, 31 mg is about the maximum amount of material that can be loaded on a column of this size. The column was eluted with 100 ml of concanavalin A buffer while collecting 1-ml fractions. The column was further eluted with 200 ml of a 4% (w/v) solution of α-methyl-D-mannopyranoside (Sigma) in concanavalin A buffer. One peak of carbohydrate-containing material was eluted from the column in the initial buffer wash. The fractions in the void peak, which contained better than 80% of the elicitor activity applied to the column, were pooled, evaporated to about 3 ml and desalted on a Bio-Gel P-2 (Bio-Rad) column (2.5 × 11.5 cm). This

TABLE I

Neutral sugar composition and relative elicitor activity of the elicitor-active fractions obtained during the preparation of the yeast extract elicitor

FRACTION	NEUTRAL SUGAR COMPOSITION[a]					RELATIVE ELICITOR ACTIVITY[b]	% YIELD OF GLUCAN
	ARABINOSE	GALACTOSE	RIBOSE	MANNOSE	GLUCOSE		
Dialyzed 80% Ethanol Precipitate	2.2	0.7	9.5	80.0	7.6	1	100
60% Ethanol Supernatant	2.8	0.7	11.4	74.1	11.0	1	45
DEAE-Cellulose Eluent	2.6	1.0	0	87.0	9.3	6	20
Sulfopropyl-Sephadex Eluent	3.0	1.0	0	84.9	11.1	3	14
Concanavalin-A Sepharose Eluent	2.8	1.5	0	1.0	94.7	100	8

[a] Mole % compositions as determined by the alditol acetate method (18).

[b] Calculated as described in Ayers et al. (6). An A₂₈₉=0.5 was arbitrarily chosen as the point of comparison. The data were normalized to the activity of the concanavalin-A Sepharose eluent.

final step yielded 1.93 mg of carbohydrate. The material which eluted from the concanavalin A-Sepharose column with α-methyl-D-mannopyranoside was evaporated to about 60 ml under reduced pressure at 40 C and dialyzed extensively against deionized H_2O.

The elicitor-active material which had no affinity for the concanavalin A-Sepharose column was composed almost exclusively of glucose with small amounts of mannose, galactose, and arabinose (Table I). This material was an extremely effective elicitor of glyceollin accumulation as determined by the cotyledon assay (Fig. 1) and had a relative elicitor activity 100 times greater than that of the crude 80% ethanol precipitate. The material which bound to the concanavalin A-Sepharose column and which was eluted by α-methyl-D-mannopyranoside possessed a small amount of glucose but had a neutral sugar composition similar to that of the two mannan-rich fractions retained by the DEAE-cellulose column. This fraction possessed a relative elicitor activity of 3.

CHARACTERIZATION OF THE ELICITOR

Elicitor Activity. A glucan in an autolysate of yeast possesses the ability to elicit phytoalexin (glyceollin) accumulation in soybean tissues. A mannan, the quantitatively dominant polysaccharide of the lysate, does not possess elicitor activity. This is illustrated in Figure 1 which compares the elicitor activities of three yeast lysate fractions: the crude 80% (v/v) ethanol precipitate (glucose:mannose = 1:6); the purest mannan obtained (from the DEAE-cellulose column, glucose:mannose = 1:51); and the purest glucan obtained (from the concanavalin A-Sepharose column, glucose:mannose = 100:1). The other purified mannan-containing fractions possessed elicitor activity curves which closely resembled that of the mannan illustrated in Figure 1. The abilities of the elicitors isolated from yeast and from *P. megasperma sojae* to stimulate glyceollin accumulation in both cotyledons and hypocotyls of soybeans are similar. A comparison of the activity of these two elicitors on the two soybean tissues is given in Figure 2.

The heat stability of the yeast and *P. megasperma sojae* elicitors was demonstrated by autoclaving at 121 C for 30 min. This treatment does not affect the activity of either elicitor (data not presented).

Sugar and Glycosyl Linkage Compositions of Yeast Elicitor. The mole per cent compositions of fractions from various stages of the purification of the yeast elicitor are shown in Table I. A gas
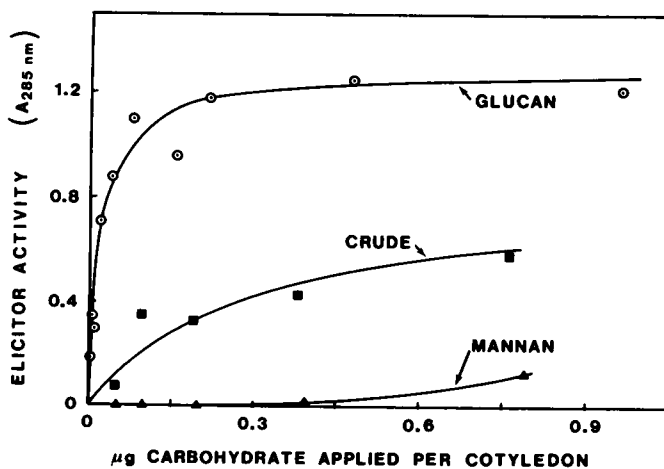

FIG. 2. Comparison of the activities of the yeast (▲——▲) and the *P. megasperma* var. *sojae* (fraction I) (⊙——⊙) elicitors. A: cotyledon assay (5): 75-μl samples were applied to each of 10 cotyledons. Each datum in the figure represents the average of two such sets of 10 cotyledons. Both the yeast and *Phytophthora* elicitors were assayed on the same day using the randomized cotyledons from a single batch of plants. The A_{285} of the wound droplet solution is used as a measure of elicitor activity. B: hypocotyl assay (5): 20-μl samples were applied to each of 10 wounded hypocotyls. Both the yeast and *P. megasperma* var. *sojae* elicitors were assayed on the same day using the same batch of plants. The dried ethanol extracts of the wounded hypocotyl sections were redissolved in methanol and applied to a preparative thin layer silica gel plate. The thin layer plate was then developed vertically in hexane-ethylacetate-methanol (60:40:5, v/v/v). The spot having the same R_F as as a glyceollin standard was scraped off the plate and extracted with 5 ml of ethanol. This material probably contains all three biologically active isomers of glyceollin (25). The A_{285} of the ethanol extract is used as a measure of elicitor activity.

chromatogram of the partially methylated alditol acetates derived from the purest elicitor isolated from yeast extract is shown in Figure 3. For comparison, a gas chromatogram of the partially methylated alditol acetates (B. S. Valent and P. Albersheim, unpublished) derived from exo-glucanase degraded *P. megasperma sojae* elicitor (7) is also shown in Figure 3. The exoglucanase treatment of the *P. megasperma* var. *sojae* glucan decreases the size of this elicitor to a size roughly comparable to the yeast elicitor (7). This enzyme does not attack the yeast glucan and does not affect the elicitor activity of either glucan (7, and unpublished results of B. S. Valent and P. Albersheim). The amounts of the glycosyl derivatives illustrated in Figure 3 are summarized in Table II.

Gel Chromatography. The size distribution of the polysaccharides present in the purest yeast glucan elicitor was determined by gel permeation chromatography. About 1.2 mg of the elicitor was applied in 1.5 ml to a Bio-Gel A-0.5m (Bio-Rad) column (1.3 × 70 cm) which had been equilibrated with 50 mM NaCl. The column was eluted with 50 mM NaCl and 1-ml fractions were collected. The fractions were assayed for carbohydrate using the anthrone assay (15). Elicitor activity was determined by the coty-

FIG. 1. Elicitor activity, in the cotyledon assay (5), of three fractions obtained during the purification of yeast extract elicitor. An aliquot (75 μl) of each sample was applied to each of 10 cotyledons. Each data point represents the average of two such sets of 10 cotyledons The amount of carbohydrate present in each fraction was calculated by the combined anthrone-alditol acetate method. The fractions are: crude 80% ethanol precipitate (■——■); mannan eluted from the DEAE-cellulose column (▲——▲); and glucan which voids the concanavalin A-Sepharose column (⊙——⊙).
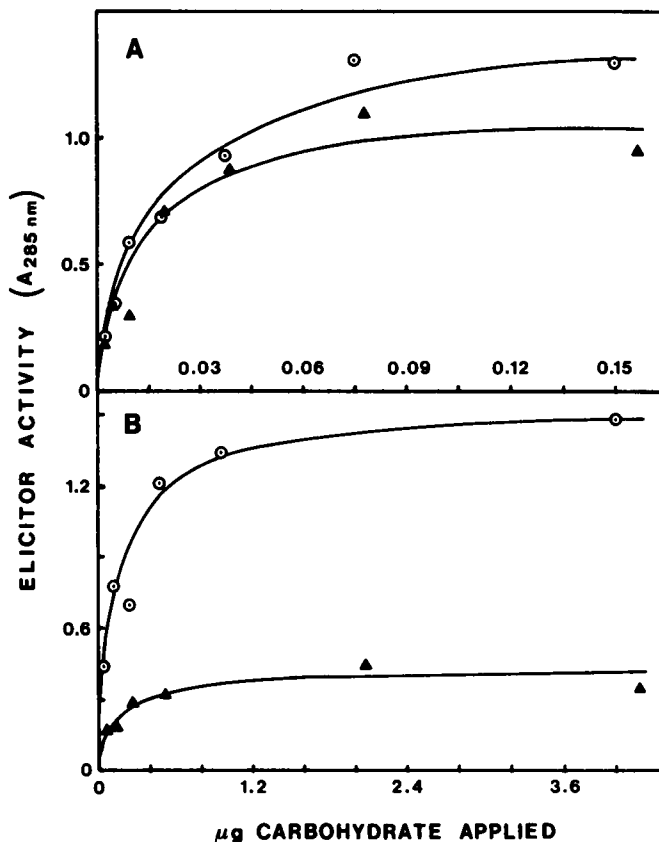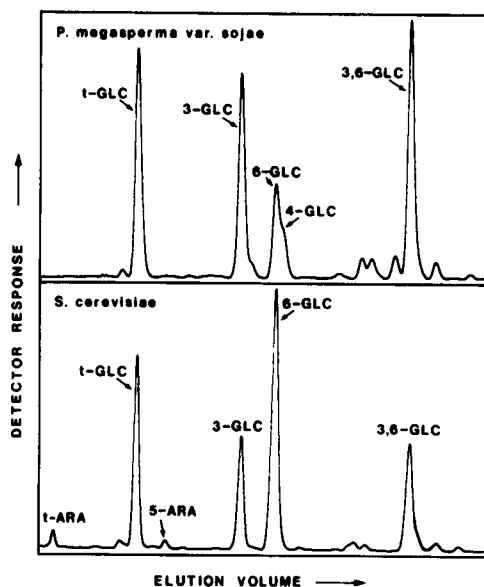
FIG. 3. Computer-drawn comparison of the gas chromatograms of the partially methylated alditol acetates derived from the yeast glucan and from the exoglucanase degraded *P. megasperma* var. *sojae* glucan. The linkages to each sugar derivative are indicated by numerical prefixes: thus, 3,6-Glc indicates that other glycosyl residues are glycosidically linked in the polysaccharide to the 3 and the 6 carbons of these glucosyl residues. Terminal glycosyl residues, glycosyl residues which have no other glycosyl residues attached to them, are indicated by t- (*e.g.* t-Glc).

TABLE II

Glycosyl linkage compositions of the yeast elicitor and exo-glucanase degraded P. megasperma var. sojae Fraction I elicitor.

Glycosyl Residue[a]	% OF TOTAL GLYCOSYL RESIDUES	
	Yeast Elicitor[b]	P. megasperma var. sojae Elicitor[c]
t-Glc	21.4	27.8
3-Glc	16.4	26.0
4-Glc	1.1	5.9
6-Glc	43.6	10.3
3,6-Glc	15.5	30.0
t-Ara	1.4	0
5-Ara	0.6	0

[a] Abbreviations: Glc = glucose, Ara = arabinose The assignments of the glycosyl residues are similar to those in Fig. 3.

[b] Obtained from the concanavalin-A Sepharose column.

[c] Exo-glucanase treated Fraction I elicitor prepared as described in Ayers et al. (7).

ledon assay. The void and included volumes were determined using blue dextran (Sigma) and glucose, respectively. The profile of carbohydrate and elicitor activity as it eluted from the Bio-Gel A-0.5m column is presented in Figure 4.

DISCUSSION

Brewers' yeast possesses a powerful elicitor of glyceollin accumulation in soybean tissue. The yeast extract was fractionated into a number of carbohydrate-containing compounds, most of which were able to stimulate the accumulation of glyceollin in soybean tissue. As with the *Phytophthora* elicitor (6), the relative elicitor activity of each carbohydrate-containing fraction is roughly proportional to the amount of glucan present in the fraction (Table I, Fig. 1, and unpublished results of the authors).
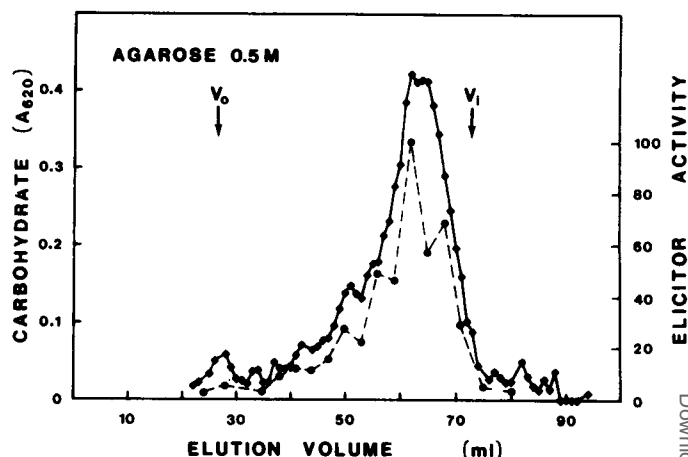

FIG. 4. Chromatography of the purified yeast elicitor on Bio-Gel A-0.5m. Yeast elicitor (1.2 mg), obtained from the concanavalin A-Sepharose column, was applied to a Bio-Gel A-0.5m column (1.3 × 70 cm) and the column eluted with 50 mM NaCl. The void volume (V_0) for this column was 27 ml and the inclusion volume (V_i was 73 ml. One-ml fractions were collected. The carbohydrate content (◆——◆) was determined by the anthrone procedure (15). The elicitor activity (●——●) of these fractions was determined by applying aliquots containing about 15 ng of carbohydrate to wounded cotyledons. This amount of elicitor results in half of the maximum level of glyceollin accumulation (Fig. 2). The elicitor activity of each fraction was normalized to that of fraction 62. Fraction 62 possessed the most carbohydrate and the most elicitor activity.

The yeast glucan elicitor shares many characteristics with the glucan elicitor which has been isolated from *P. megasperma sojae* (6, 7). These characteristics include the fact that the yeast elicitor can be separated from other carbohydrate-containing macromolecules using essentially the same techniques that were successful in isolating the *P. megasperma sojae* elicitor. A comparison of the glycosyl linkage compositions shows the presence of the same linkages in both elicitors: 3-linked glucose, 4-linked glucose, 6-linked glucose, and 3,6-linked glucose (Fig. 3 and Table II). Elicitation of glyceollin in the cotyledons and hypocotyls of soybeans requires exactly the same amounts of the yeast and *Phytophthora* glucans (Fig. 2). Thus, 15 ng of carbohydrate applied/cotyledon or 100 ng applied/hypocotyl is sufficient to result in half-maximum glyceollin accumulation in these tissues. Both the yeast and *P. megasperma sojae* elicitors are heat-stable and size-heterogeneous (Fig. 4).

There are some differences between the yeast and *Phytophthora* elicitor-active glucans. The yeast glucan is enriched in 6-linked glucosyl residues, whereas the *Phytophthora* glucan is enriched in 3-linked glucosyl residues (Table II). The yeast elicitor has relatively fewer 4-linked and 3,6-linked glucosyl residues than does the *Phytophthora* elicitor. Another difference between the yeast and *P. megasperma sojae* elicitors is the lower maximum level of phytoalexin production exhibited by the yeast elicitor. This difference is not great in the cotyledon assay, the yeast elicitor giving about 80% of the maximum response observed when the *Phytophthora* elicitor is applied (Fig. 2A). However, this difference is marked in the hypocotyl assay, where the yeast glucan results in only about 25% of the phytoalexin accumulation elicited by saturating levels of the *Phytophthora* glucan (Fig. 2B). The differences in the response of the soybean tissues to the two glucans were consistently observed. The phenomenon may result from different rates of degradation of the two elicitors by glucanases present in the plant tissues (1, 11, 19, and unpublished results of K. Cline and P. Albersheim).

The discovery in yeast extract of a potent elicitor of phytoalexin production in soybeans delineates further the role that phytoalexins play in plant disease resistance. The hypothesis has been put forward that phytoalexin production is a specific response by the

plant to invading pathogens (20). Lisker and Kuć (23) have shown that a large number of fungi having glucans in their walls are able, when applied to potato tuber discs, to stimulate the production of rishitin and other potato phytoalexins. Most of these organisms are not pathogenic on potatoes. Other work in our laboratory has shown that the soybean phytoalexin, glyceollin, is able to inhibit the growth of a spectrum of microorganisms including *S. cerevisiae* (2, 3).

The yeast extract, from which the elicitor in the present study was isolated is prepared from an autolysate of brewers' yeast. This fungus is not a known pathogen of any plant. Yet, when whole yeast are applied to soybean tissue, the plant recognizes the presence of this fungus and responds by producing growth-inhibiting phytoalexins (unpublished results). The yeast cell wall contains a structural glucan (28) which appears to be very similar in structure to the elicitor-active glucan isolated in this study. In fact, a wide variety of fungi have glucans in their mycelial walls which appear to be structurally related to the one isolated here (8). In view of the results presented in this study, other results of this laboratory (3, 6, 16, 31), and the results of others (23), one can speculate that plants, in general, can recognize such glucan elicitors in the walls of a wide variety of fungi whether or not they are pathogens, and respond by producing growth-inhibiting phytoalexins. Indeed, elicitation of phytoalexins seems to be a method by which plants ward off nonpathogens. The few microorganisms that are, in fact, pathogens must have evolved ways to overcome this mechanism of defense in their hosts.

Not all microorganisms have a glucan, such as those discussed above, in their cell walls. Gram-negative bacteria, for example, are not known to have, in their walls, glucans structurally related to the *P. megasperma* var. *sojae* and yeast elicitor-active glucans. Yet, plants produce phytoalexins when confronted with both pathogenic and nonpathogenic Gram-negative bacteria (21, 26, 27, 29, and unpublished results). It will be interesting to identify other types of nonspecific elicitors in the Gram-negative bacteria and in other organisms which do not possess glucan elicitors.

Recent reports on the anticancer activity of yeast glucan in animals (9, 14) and the ability of such glucans to activate defense mechanisms in crayfish (30) indicate that these mycelial wall components may serve as recognition determinants in a large range of organisms.

The results presented here indicate that a word of caution is in order. Yeast extract is widely used in culture media. Ebel *et al.* (16) have shown that the *P. megasperma sojae* elicitor causes dramatic changes in the metabolism of suspension-cultured soybean cells. This fact, together with the apparent interspecies activity of elicitors, makes it imperative that elicitor-containing yeast extract not be used in the media of plant tissue cultures without determining that the yeast extract has no undesirable effect on the cultures in question.

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