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Phytoalexin Induction in French Bean[1]

INTERCELLULAR TRANSMISSION OF ELICITATION IN CELL SUSPENSION CULTURES AND HYPOCOTYL SECTIONS OF *PHASEOLUS VULGARIS*

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RICHARD A. DIXON, PRAKASH M. DEY, MICHAEL A. LAWTON[2], AND CHRISTOPHER J. LAMB[3]
Department of Biochemistry, Royal Holloway College, University of London, Egham Hill, Egham, Surrey TW20 0EX United Kingdom (R. A. D., P. M. D.); and Department of Biochemistry, University of Oxford, Oxford 0X1 3QU United Kingdom (M. A. L., C. J. L.)

ABSTRACT

Treatment of hypocotyl sections or cell suspension cultures of dwarf French bean (*Phaseolus vulgaris* L.) with an abiotic elicitor (denatured ribonuclease A) resulted in increased extractable activity of the enzyme L-phenylalanine ammonia-lyase. This induction could be transmitted from treated cells through a dialysis membrane to cells which were not in direct contact with the elicitor. In hypocotyl sections, induction of isoflavonoid phytoalexin accumulation was also transmitted across a dialysis membrane, although levels of insoluble, lignin-like phenolic material remained unchanged in elicitor-treated and control sections. In bean cell suspension cultures, the induction of phenylalanine ammonia-lyase in cells separated from ribonuclease-treated cells by a dialysis membrane was also accompanied by increases in the activities of chalcone synthase and chalcone isomerase, two enzymes previously implicated in the phytoalexin defense response. Such intercellular transmission of elicitation did not occur in experiments with cells treated with a biotic elicitor preparation heat-released from the cell walls of the bean pathogen *Colletotrichum lindemuthianum*. The results confirm and extend previous suggestions that a low molecular weight, diffusible factor of host plant origin is involved (in French bean) in the intercellular transmission of the elicitation response to abiotic elicitors.

Phytoalexins are "low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms" (21). Their synthesis and accumulation may also be induced by treatment of plant tissues with fungal cell wall components (biotic elicitors) (1) and a wide range of unnatural abiotic elicitors including heavy metal salts (12), surfactants (13), and basic polypeptides such as denatured RNase A (6). Detailed physiological studies have led to the proposal that induction of phytoalexin accumulation by these various agents involves local host cell damage or death, causing release of a plant metabolite(s) which stimulates synthesis of phytoalexins in surrounding healthy cells (2). It has been shown that aqueous extracts of French bean tissue stimulate the production of phytoalexins in bean hypocotyls (14) and cultured cells (15) suggesting the presence of an endogenous elicitor in plants. More recently, an endogenous elicitor activity has been demonstrated in cell extracts from soybean (*Glycine max* L.) and appears to be a pectic fragment of the cell wall (11); a pectic fragment also appears to be involved in the intercellular transmission of wound-induced increases in proteinase inhibitor levels (23). However, despite the identification of low mol wt endogenous elicitors and the demonstration of a close correlation between local cell damage and induction of phytoalexin accumulation, there is no direct evidence for host-mediated intercellular transmission of the elicitation response. In the present paper, the induction of enzyme activities related to phytoalexin production is examined in French bean cells separated by a dialysis membrane from equivalent cells directly exposed to macromolecular elicitors. A preliminary account has been published (16) of this approach which allows a direct examination of the possible involvement of host-mediated intercellular transmission of the elicitation signal in phytoalexin accumulation.

MATERIALS AND METHODS

Plant Material. Seeds of dwarf French bean (*Phaseolus vulgaris* cv The Prince) were surface-sterilized for 15 min in NaOCl (1–1.4% available Cl_2), washed with sterile distilled H_2O, and sown in moist vermiculite. Germination was for 7 d in the dark at 25°C.

Cell Suspension Cultures. Cell suspension cultures of *P. vulgaris* (cv Canadian Wonder) were grown in a modified Schenk and Hildebrandt medium as described previously (8). In all the experiments described, cells were in mid-growth phase (6–7 d after subculture).

Elicitors. Bovine pancreatic RNase A (EC 3.1.4.22) was purchased from Sigma Chemical Company. It was denatured by autoclaving in distilled H_2O for 15 min at 120°C. *Colletotrichum lindemuthianum* (race β and Commonwealth Mycological Institute isolate IMI 112166) was maintained on a semisolid glucose-neopeptone medium (19). The growth of liquid cultures of the fungus, the isolation of mycelial walls, and the release of crude elicitor preparations by heat treatment were as described (8).

Preparation of Bean Hypocotyl Extract. Hypocotyls were excised from 7-d-old etiolated seedlings grown as described above. The hypocotyls (100 g) were autoclaved, with no added H_2O, for 15 min at 120°C. After cooling, the juice (10 ml) released from the collapsed tissue was decanted under aseptic conditions and stored at −20°C until required.

Treatment of Hypocotyl Sections for Demonstration of Transmissible Elicitor Activity. Etiolated hypocotyl segments (5 cm in length for determination of isoflavonoid and lignin levels, 2 cm in length for determination of enzyme activities) were longitudinally bisected with a sterile razor blade. Sets of half hypocotyls were

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[2] Present address: Department of Biology, Washington University, St. Louis, MI 63130.

[3] Present address: The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

placed, cut side uppermost, on moist filter paper in Petri dishes (10 segments/dish). Sterile distilled H_2O was applied to the cut surfaces and a moist dialysis membrane placed on top. The upper surface of the membrane was carefully flooded with an aqueous solution of denatured RNase A (0.5 $mg \cdot ml^{-1}$), and the top halves of the hypocotyls were placed on the membrane, with their cut surfaces downwards, exactly above their corresponding lower halves. Filter paper moistened with sterile distilled H_2O was then packed on top of the hypocotyls in order to keep the halves pressed together; the Petri dishes were then sealed with polythene and the samples incubated in the dark at 25°C for 8 h (for analysis of PAL[4] activity) or 48 h (for isoflavonoid and lignin determination). Three control experiments were included; these were: (a) as above, but with sterile distilled H_2O replacing the RNase solution on the upper surface of the membrane; (b) with no hypocotyl sections above the dialysis membrane, the upper surface of the membrane being moistened with sterile H_2O; and (c) as in (b), but with RNase solution replacing the H_2O. Sets of treated hypocotyl sections were separated into upper and lower halves, weighed, frozen in liquid N_2, and stored at −20°C until required for analysis of PAL, isoflavonoids, or lignin.

Treatment of Cell Suspension Cultures for Demonstration of Transmissible Elicitor Activity. All experiments were performed with 50-ml batches of suspension culture in 150-ml conical flasks. A small dialysis bag containing 10 ml of cell suspension from the same culture batch was added to each flask immediately prior to the addition of elicitor to the external cells. The dialysis bags had been sterilized by autoclaving in distilled H_2O and were sealed with sterile plastic clips. In addition to 'minus elicitor controls', each experiment was performed with a set of controls in which the external cell suspension was replaced with conditioned medium (prepared by removal of cells from suspensions of the same batch as used in the test experiments) containing elicitor; conditioned medium controls would indicate any production of low mol wt diffusible elicitors resulting from the action of plant enzymes, present in the culture medium, on the exogenously added macromolecular elicitor. After the required incubation period, cells from inside and outside the dialysis bags were harvested by suction filtration, frozen in liquid N_2, and stored at −20°C until required for enzyme assays.

Extraction and Assay of Enzymes. All enzyme assays were performed with crude extracts. Extraction and assay of PAL, CHS, and CHI were as previously described (8).

Extraction and Determination of Isoflavonoids. The hypocotyl sections from each treatment were further bisected by cutting longitudinally, at 90 degrees to the previously cut surface, with a razor blade. One set of halves was used for determination of isoflavonoids, the other for determination of lignin. Batches of hypocotyl sections were extracted in a pestle and mortar with 20 ml ethanol and samples were prepared for TLC analysis of isoflavonoids as described elsewhere (26). Chromatograms (Machery-Nagel silica gel G/UV_{254}) were developed in chloroform:ethanol (100:5, v/v). Isoflavonoids were eluted from chromatograms in 1 ml ethanol. Phaseollin (R_F 0.58), kievitone (R_F 0.12), and 2'-hydroxygenistein (R_F 0.20) were quantitatively determined from their reported extinction coefficients at λ_{max} 280, 293, and 260 nm, respectively (26). Kievitone and 2'-hydroxygenistein were further characterized by the bathochromic shifts in their UV spectra following addition of ethanolic $AlCl_3$, and 2'-hydroxygenistein additionally identified by comparison of chromatographic and spectroscopic properties with an authentic sample. An unidentified isoflavone (R_F 0.40, λ_{max} 266 nm) was eluted from chromatograms as above and its concentration expressed as A_{266} per g fresh weight.

[4] Abbreviations: PAL, L-phenylalanine ammonia-lyase (EC 4.3.1.5); CHS, chalcone synthase; CHI, chalcone isomerase (EC 5.5.1.6).

Determination of Insoluble Phenolic Material. Lignin-like material was determined by a modification of the method of Stafford (24). Bisected hypocotyl sections were dried to constant weight in an oven at 60°C and were then successively extracted with diethyl ether, H_2O (three times), and 50% methanol, the residue being hydrolyzed for 16 h in 1 M NaOH at 70°C. After centrifuging and washing the pellet, the combined supernatants were adjusted to pH 8.0 and equal aliquots added to 3 ml of 50 mM NaOH and 3 ml of 10 mM NaH_2PO_4 (pH 7.0) in quartz cuvettes, for the measurement of difference spectra (ΔE, pH 12.3–7.0). The amount of alkali-released phenolic material was estimated from the peaks in the difference spectra at 245 and 300 nm.

RESULTS

Transmission of Elicitation in Split Hypocotyl Sections. PAL activities of about 1.5 pkat/2 cm segment were observed when split hypocotyl sections were incubated for 8 h in contact with a moistened dialysis membrane (Table I). This value is approximately twice that observed in intact, unbisected hypocotyls, the increase being a response to wounding. Treatment of the upper hypocotyl halves with RNase resulted in an approximately 70% increase above the wounded control activity, and a corresponding increase in activity was also seen in the lower hypocotyl halves which were not in direct contact with the abiotic elicitor. No enzyme induction was observed in controls with elicitor but no hypocotyl sections above the membrane.

A generally similar pattern of induction by RNase, and transmission of the response, was observed with respect to isoflavonoid levels in the hypocotyl sections 48 h after treatment (Table I). Approximately 100% increases in the levels of the isoflavones 2'-hydroxygenistein and isoflavone 266, the isoflavanone kievitone, and the pterocarpan phaseollin were observed in hypocotyl sections directly treated with RNase as compared to control sections; in the corresponding lower sections, increased phytoalexin levels were also observed. However, smaller but significant increases in phytoalexin levels were also seen in control lower half sections with RNase but no plant material above the dialysis membrane. That this was not the result of RNase passing through the membrane was suggested by the similar values for isoflavonoid levels observed in control sections where H_2O replaced RNase above the dialysis membrane.

The difference spectra for wall-bound phenolic material from both H_2O-treated and RNase-treated hypocotyl sections exhibited maxima at 245 and 300 nm. Identical spectra were obtained for all induced and control samples, and no significant quantitative or qualitative differences in wall-bound phenolic material were observed in the various treatments (Table I).

The above results strongly suggest that a diffusible component with the ability to induce isoflavonoid phytoalexins and PAL activity is released by bean cells following treatment with denatured RNase. However, split hypocotyls may not be the best system to use in order to demonstrate this phenomenon, as high background increases in PAL (16, 26) and phytoalexin levels (26) are observed in response to wounding in the absence of elicitor. As a result of the wound-induced increases in enzyme level, a strict correlation between absolute enzyme activities and phytoalexin levels may not be obtained (26), and the overall effect of elicitor treatment appears less striking than in tissues which exhibit a much lower wound response (*e.g.* soybean [1]). Furthermore, it is possible that the extent of the wound response may differ depending on whether or not the sections are in close contact with other tissue, as suggested by the elevated phytoalexin levels in controls with no plant tissue above the dialysis membrane. To circumvent these problems, further investigations of the transmission of the elicitation signal were performed using liquid cell suspension cultures.

Transmission of Elicitation in Cell Suspension Cultures Treated

Table I. *Transmission of Elicitation between Split Hypocotyl Sections of P. vulgaris*

Treatment (see Text)[a]	PAL Activity at 8 h		Isoflavonoid Levels at 48 h								Lignin Difference Spectrum at 48 h[b]	
	Exp. 1	Exp. 2	Kievitone		2′-Hydroxygenistein		Phaseollin		Isoflavone$_{266}$[c]		λ_{245}	λ_{300}
			Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2		
	pkat/segment		*nmol g^{-1} fresh wt*									
$\frac{H_2O + \text{hypocotyl sections}}{H_2O + \text{hypocotyl sections}}$	1.46	1.38	21.3	21.7	32.9	25.2	27.4	26.4	0.15	0.15	35.6	14.2
	1.59	1.47	19.7	21.0	41.0	38.1	20.0	19.2	0.15	0.24	31.1	13.3
$\frac{\text{RNase + hypocotyl sections}}{H_2O + \text{hypocotyl sections}}$	2.26	2.17	55.3	59.1	83.9	72.0	60.1	57.9	0.38	0.45	31.0	11.7
	2.53	2.41	42.1	45.0	73.4	63.0	40.5	36.9	0.33	0.48	35.0	13.7
$\frac{\text{RNase}}{H_2O + \text{hypocotyl sections}}$	1.53	1.28	33.0	38.1	55.9	50.4	31.1	30.0	0.24	0.14	35.7	16.5
$\frac{H_2O}{H_2O + \text{hypocotyl sections}}$	1.55	1.49	30.1	31.5			30.5	28.9				

[a] The diagrams refer to the arrangement of hypocotyl sections above and below the dialysis membrane (horizontal line). The test solutions (RNase or H_2O) were placed between the tissue and the membrane.

[b] One aliquot of an alkaline extract from a crude cell wall preparation was adjusted to pH 7.0, another to pH 12.0; the values represent the difference in extinction ($\Delta E_{1\,cm}$ pH 12.0–7.0) at 245 and 300 nm expressed per g dry weight of initial hypocotyl sections extracted.

[c] Results expressed as *A* at 266 nm per g fresh weight.

with an Abiotic Elicitor. As previously observed (7), denatured RNase A (0.5 mg ml^{-1}) was a potent elicitor of PAL induction in cell suspension cultures of *P. vulgaris* (Fig. 1A, ●). Approximately 2 h after the onset of increased PAL appearance in cell cultures directly exposed to the elicitor, parallel increases in extractable PAL activity were observed in cells separated from the elicitor by being inside a dialysis bag (Fig. 1A, ■). No enzyme induction was observed in the cells inside the bag if the external medium consisted of cell culture minus elicitor or conditioned medium plus elicitor. Similar induction and transmission of the elicitation response were observed with respect to the enzymes CHS and CHI (Fig. 1, B and C), although in these cases the induced activities of the enzymes inside the bag, over the time period studied, reached only 21% and 38%, respectively, of the maximum-induced activities of the external cells, as opposed to over 90% in the case of PAL.

Maximum PAL induction in the suspension cultures was observed at a final RNase concentration of 0.5 mg·ml^{-1} culture. This concentration also resulted in maximum transmitted induction in the cells inside the dialysis bag.

These results complement the data obtained with the split hypocotyl sections, and further suggest the release of a low mol wt elicitor component from the bean cells following treatment with RNase. The lack of transmission of elicitation in controls where cells were separated by the dialysis membrane from RNase plus conditioned medium indicates that (a) intact RNase does not pass through the dialysis membrane and (b) the low mol wt elicitor could not arise by degradation of RNase by extracellular proteolytic enzymes. However, such controls could not discount the possibility of RNase breakdown at the surface of the cultured cells, with subsequent release of elicitor-active low mol wt peptides. To further investigate this possibility, the effects of proteolytically digested RNase preparations on PAL induction in the cell cultures were examined (Table II). Degradation of RNase by trypsin, chymotrypsin, and pronase resulted in the production of a large number of different low mol wt peptides, as assessed by chromatography on Sephadex G-50 (results not shown). However, of the various treatments used, only high concentrations of intact RNase exhibited significant elicitor activity. It is therefore concluded that the transmissible elicitor is not a breakdown product of the RNase.

PAL Induction in Cell Suspension Cultures Treated with Biotic Elicitors. Biotic elicitor preparations heat-released from the cell walls of *C. lindemuthianum* isolate IMI 112166 and race β caused marked induction of PAL activity in the *P. vulgaris* cell suspension cultures (9, 18; Fig. 2, A and B, ●). However, no induction of the enzyme was observed in cells separated from the elicitor-treated cells by a dialysis membrane (Fig. 2, A and B, ■). In preliminary experiments, some transmissible elicitation was observed. This, however, was shown to be due to the presence of low mol wt components in the elicitor samples, presumably resulting from either incomplete dialysis of the elicitor during preparation or breakdown during storage. These effects were not observed with fresh, exhaustively dialyzed preparations. This lack of intercellular transmission was observed at all concentrations of biotic elicitor tested (0–200 μg glucose equivalents ml^{-1}).

Induction of PAL in Cell Suspension Cultures by an Extract from *P. vulgaris* Hypocotyls. The crude exudate from autoclaved bean hypocotyls induced PAL activity in the cell suspension cultures (Fig. 3, ●). Induction of phytoalexin accumulation in *P. vulgaris* cell suspension cultures by a similar preparation had been reported previously (15). Although PAL was also induced in the cells inside the dialysis bag in our experiments, this induction occurred irrespective of the presence of bean cells in the external medium (Fig. 3). In contrast to the results obtained using RNase, there was no lag between the onset of increased PAL appearance in the cells inside the dialysis bag and in those in the external medium in response to the hypocotyl extract.

DISCUSSION

The above results confirm and extend previous suggestions that low mol wt diffusible factors of host plant origin may be involved in transmission of the host's response to abiotic elicitors in several plant species. Indirect evidence for the involvement of such factors has come from experiments in which (a) aqueous extracts from either dead or living French bean tissue stimulated the production of phytoalexins in bean hypocotyls (14) or cell suspension cultures (15); (b) limited cell death at the surface of French bean cotyledons resulting from treatment with chloroform or surfactants was followed, 15 to 20 h later, by the accumulation of phytoalexins, this

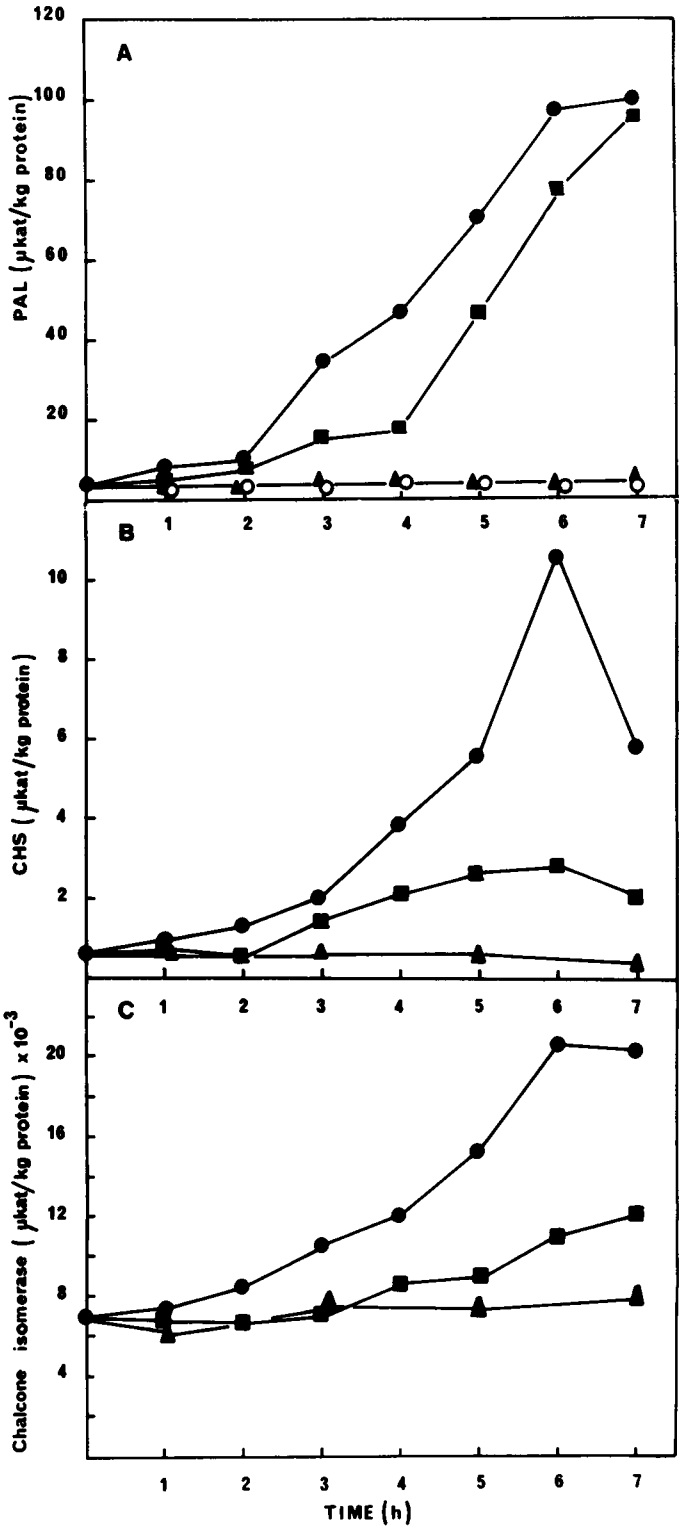

FIG. 1. Induction of PAL, CHS, and CHI activities in French bean cell suspension cultures following addition of RNase A (0.5 mg·ml^{-1}) to cell suspension, or conditioned culture medium alone, containing a smaller batch of cell suspension within a dialysis bag. Enzyme activities were measured in: (a) cells outside the bag which were directly in contact with RNase (●); (b) cells inside the bag surrounded by external cells plus RNase (■); (c) cells inside the bag surrounded by external conditioned medium plus RNase (▲); (d) cells inside the bag surrounded by external untreated cells (○).

Table II. *Effects of Intact and Degraded RNase Preparations on PAL Activities in Phaseolus vulgaris Cell Suspension Cultures*

Batches of denatured RNase A (10 mg in 1 ml H_2O, pH 8.0) were incubated with 1 mg trypsin, chymotrypsin, or pronase for 16 h at 30°C. The extent of degradation of the RNase by the protease treatments was assessed by comparison of the elution patterns of the intact and degraded enzymes following gel-filtration chromatography on Sephadex G-50 (see "Results"). Samples of digested and intact RNase were added, at the final concentrations shown, to 10-ml batches of cell suspension culture, and cells were harvested for assay of PAL after 7 h incubation. Control solutions containing protease but no RNase failed to induce PAL above the basal level obtained with H_2O alone.

Addition to Cell Culture	Final Concn.	PAL Activity at 7 h
	mg/ml culture	*μkat/kg protein*
H_2O		7.5
RNase	0.50	86.7
RNase	0.25	19.2
RNase	0.10	15.0
Trypsin-digested RNase	0.50	10.1
Chymotrypsin-digested RNase	0.50	10.8
Pronase-digested RNase	0.50	9.0

accumulation occurring only if a substantial proportion of living cells remained in close association with the dying cells (2); and (c) $HgCl_2$ treatment of pea cotyledons resulted in the release of components with elicitor activity (12). The results reported in the present paper directly indicate that a low mol wt endogenous elicitor is released following treatment of French bean cells with an abiotic elicitor. Of particular importance for future studies is the fact that, in suspension cultures, the endogenous elicitor is released into the culture medium, from where it may presumably be readily extracted and purified, by a treatment which does not involve the harsh conditions previously used for release of endogenous elicitor from intact hypocotyls (15) or plant cell walls (11). Furthermore, no wounding of the tissue is required in this system.

In the present work, measurements of PAL induction in cell suspension cultures clearly demonstrate that endogenous elicitor activity is rapidly released from abiotic elicitor-treated cells within the first 2 h of treatment (Fig. 1). Increased PAL and CHS induction, resulting from increased rates of their *de novo* synthesis, precede accumulation of isoflavonoid phytoalexins in French bean cell suspension cultures (7, 9, 17, 18), and changes in PAL and CHS synthetic rate are among the earliest responses to elicitor measured in this system (9, 17). CHS and CHI are enzymes involved specificially in the synthesis of flavonoids/isoflavonoids, whereas the flux through the PAL reaction may also be channeled into the formation of lignin and other phenylpropanoids. However, in bean hypocotyls, RNase induction and transmission of the PAL response appears to be associated with isoflavonoid rather than lignin production (Table I). These results, coupled with the induction of CHS and CHI in the cell cultures, suggest that the endogenous elicitor may function in the transmission of the phytoalexin response rather than in the induction of lignification. Phytoalexin levels were not monitored in detail in the cell cultures as the overall pattern of induced isoflavonoids produced in the cultured cells in response to RNase or transmissible elicitor was different to that observed in intact bean tissues. In particular, levels of phaseollin and kievitone were low relative to several unidentified isoflavones and coumestans. Full analysis of the biological activity of the host elicitor must await the isolation and purification of the molecule(s) released by RNase treatment.

The lack of intercellular transmission of PAL induction in response to biotic preparations from *C. lindemuthianum* (Fig. 2) suggests that different mechanisms may operate for the overall transmission of the phytoalexin response to biotic and abiotic elicitors. RNase is a basic polypeptide, and is known to be highly

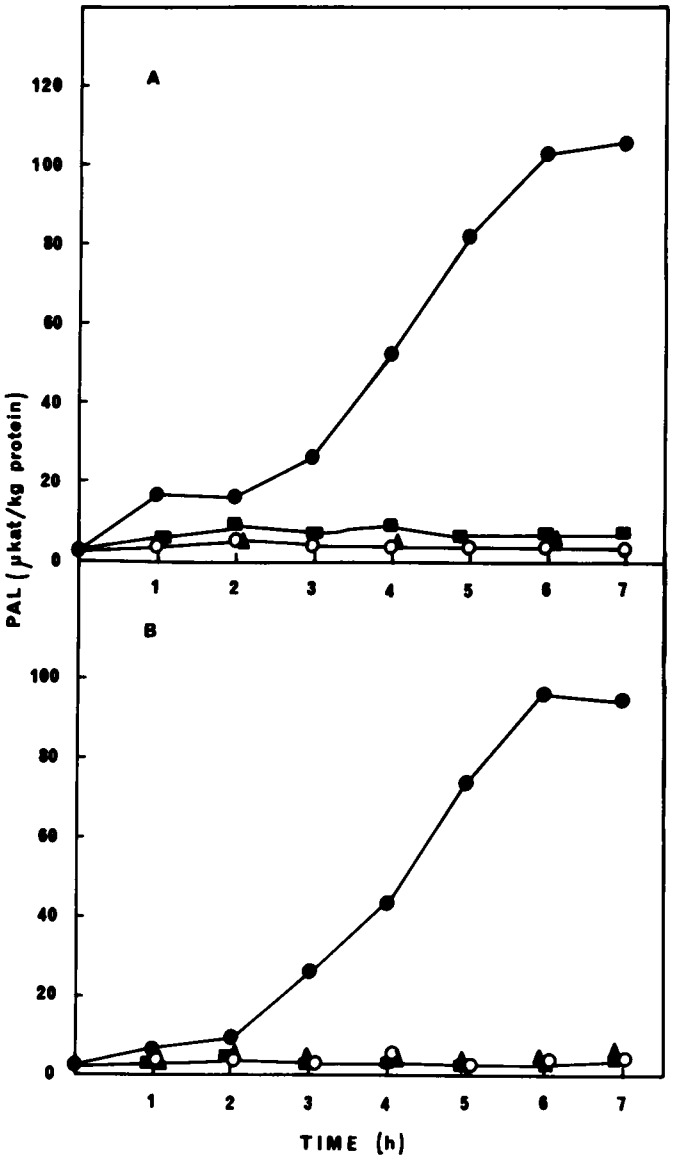

FIG. 2. Induction of PAL activity in French bean cell suspension cultures following addition of 60 μg glucose equivalents ml^{-1} of elicitor heat-released from the cell walls of *Colletotrichum lindemuthianum* race β (A) or Commonwealth Mycological Institute isolate IMI 113166 (B). Elicitor was added to cell suspension, or conditioned culture medium alone, containing a smaller batch of cell suspension within a dialysis bag. Enzyme activity was measured in: (a) cells outside the bag which were directly in contact with elicitor (●); (b) cells inside the bag surrounded by external cells plus elicitor (■); (c) cells inside the bag surrounded by external conditioned medium plus elicitor (▲); (d) cells inside the bag surrounded by external untreated cells (○).

disruptive to plant protoplasts, even when enzymically inactive (22). Other abiotic agents such as $HgCl_2$, $CHCl_3$, and surfactants also cause damage to cell membranes, and therefore have the potential to bring about release of compartmentalized metabolites which may transmit the phytoalexin response to neighboring cells. Although biotic elicitor preparations may also be phytotoxic (5, 10), recent evidence has suggested that mycelial walls or wall-released elicitors from *Phytophthora megasperma* f.sp *glycinea* may be degraded by host enzymes to yield active (27) or inactive (4) elicitor fragments, respectively; such active fragments may transmit the phytoalexin response to neighboring cells during the early stages of incompatible host-parasite interactions. A lack of pro-

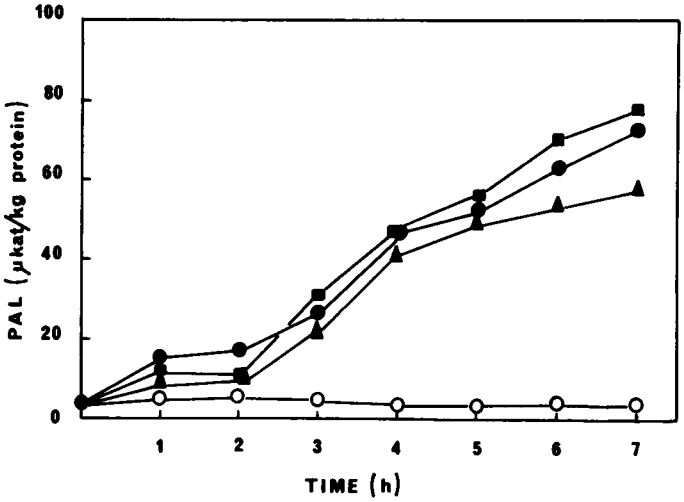

FIG. 3. Induction of PAL activity in French bean cell suspension cultures following addition of an extract from autoclaved bean hypocotyls. The extract (equivalent to 20 g hypocotyl tissue/50 ml culture) was added to cell suspension, or conditioned medium alone, containing a smaller batch of cell suspension within a dialysis bag. Enzyme activity was measured in: (a) cells outside the bag which were directly in contact with the extract (●); (b) cells inside the bag surrounded by external cells plus extract (■); (c) cells inside the bag surrounded by external conditioned medium plus extract (▲); (d) cells inside the bag surrounded by external untreated cells (○).

duction or recognition of such elicitors may characterize compatible interactions, the ultimate, late accumulation of phytoalexins in these cases perhaps resulting from cell damage/death and the subsequent involvement of released, constitutive elicitors. One implication of this hypothesis, therefore, is that the production of endogenous elicitor may be limited to compatible interactions at the time when the fungus is beginning to kill host cells. Depending upon external environmental conditions (*e.g.* temperature), the production of phytoalexins at such times may or may not be effective in partially controlling lesion limitation.

Candidates for host endogenous elicitors have included a pectic polysaccharide from the plant cell wall (11), the gaseous plant hormone ethylene (3), and biogenic amines (25). Recently, direct involvement of the two latter candidates has been discounted (20, 25). The endogenous elicitor released on autoclaving French bean hypocotyls appears to be a low mol wt neutral molecule (15), and in the present work autoclaved hypocotyl preparations induced PAL activity, with equal lag periods, in cultured cells both inside and outside the dialysis membrane, suggesting that a low mol wt component in the preparations rapidly equilibrates across the dialysis membrane, possibly without the need for release of further factors. As in the case of the proposed pectic fragment elicitor from soybean cell walls (11), autoclaving of French bean walls results in release of an active elicitor component (R. A. Dixon, unpublished observations). Studies are now in progress to determine the structure of the molecule(s) released by treatment of cell suspension cultures with RNase, and to compare them to the active components from autoclaved hypocotyl extracts and cell walls.

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