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Lipid Peroxidation Is a Consequence of Elicitor Activity[1]

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ABSTRACT

Elicitor-active preparations from the fungal pathogen of bean *Colletotrichum lindemuthianum* stimulated the accumulation of products characteristic of lipid peroxidation in treated bean tissues. Bean suspension cells treated with crude and purified elicitors accumulated 'lipofuscin-like pigment' (LFP) and malondialdehyde. The accumulation of LFP after about 6 h of treatment coincided with the onset of visible browning and production of the bean phytoalexins kievitone, phaseollin, and phaseollinisoflavan. The induction of phytoalexins and accumulation of LFP were also triggered by treatments with generators of activated oxygen species, xanthine:xanthine oxidase and Fe:ethylenediaminedi-*o*-hydroxyphenylacetic acid. These data suggest that generation of active oxygen species may be involved in lipid peroxidation triggered by elicitors.

Plants frequently express resistance to microbes by a mechanism which involves dramatic metabolic changes in the plant cells at the challenge site. This hypersensitive response is accompanied by necrosis of cells in the contact zone and by the accumulation of phenolics, such as oxidized polyphenols and low mol wt components with antibiotic activity termed phytoalexins. Hypersensitivity has also been associated with lipid peroxidation in cucumber challenged with an incompatible bacterium (14) and in cowpea infected by cucumber mosaic virus (13).

The responses that characterize hypersensitivity, such as phytoalexin production, can be triggered by treatment of plant tissues with microbial components termed elicitors. Consequently, these studies were initiated to determine whether lipid peroxidation can be triggered by elicitors and whether induction of lipid peroxidation causes phytoalexin production. Suspension cultured cells and cotyledon tissue of bean, *Phaseolus vulgaris*, were used as the test tissues. Elicitors were prepared from the fungal pathogen of bean, *Colletotrichum lindemuthianum*. Xanthine:xanthine oxidase and chelates of iron were used as generators of free radicals which promote lipid peroxidation (4, 18, 22).

MATERIALS AND METHODS

Materials. Mannitol, Tiron,[2] methionine, sodium benzoate, xanthine, fluorescein diacetate, and 1,1,3,3-tetraethoxypropane were purchased from Sigma. The enzymes xanthine oxidase (EC 1.1.3.22) from buttermilk (X1875), SOD (EC 1.15.1.1) from bovine blood (S8254), and catalase (EC 1.11.1.6) from bovine liver (C40) were also obtained from Sigma. All other chemicals were of reagent grade.

Preparation and Utilization of Fungal Elicitors. Elicitors were purified from the culture filtrate of the α-race of *C. lindemuthianum* by procedures described by Tepper and Anderson (26). These elicitors included a galactoglucomannan of approximately 60,000 D and partially purified polysaccharides which were not adsorbed by DEAE-Sephadex (26). Mycelial MSW were released from the surface of α-race mycelia by 1 M NaCl extraction as discussed in Rogers and Anderson (23).

Elicitor activity of the fungal components on cotyledons of *P. vulgaris* cv Dark Red Kidney was assayed as described by Tepper and Anderson (26). Elicitor activity was also assayed using suspension cultured cells. Suspension cultures of bean cv Dark Red Kidney, a gift from Dr. C. Lamb (Salk Institute, San Diego, CA), were maintained on medium described by Dixon et al. (7) with weekly transfer and growth at 26°C on an orbital shaker at 110 rpm in darkness. Seven d after transfer, 50 ml aliquots of the suspension cells were treated with various elicitor preparations or other chemical agents. After defined incubation times in the dark at 22°C on an orbital shaker at 110 rpm, cells were harvested by filtration, weighed, and when appropriate, stored at −80°C.

Elicitor activity was assayed by measuring phenolic accumulation in extracts prepared by grinding cells (1 g) in 10 ml of acetone and filtration of the homogenates through Whatman No. 1 (Whatman Ltd., England). The filtrate was evaporated at 50°C under an air stream and the film dissolved in 2 ml of distilled water. The solution was extracted with 5 ml of ethyl acetate, evaporated, and dissolved in 0.5 ml of 95% ethanol. Phenolics were determined in these ethanol extracts by measuring absorbance at 280 nm with salicylic acid as the standard (26). The bean phytoalexins, phaseollin, phaseollinisoflavan, and kievitone, were identified in the extracts by TLC on alumina plates (Eastman, 13252) with chloroform:methanol (100:4) as the solvent and a 10-cm running front. Detection of the phytoalexins on the plate involved visualization by UV light, bioassay with *Cladosporium cucumerinum* (5), or spraying with phosphomolybdate (5 mg/ml in ethanol) and heating at 100°C for 30 min. R_F values (× 100) were 2, 28, and 48 for kievitone, phaseollinisoflavan, and phaseollin, respectively. Quantification of the phytoalexins was performed by HPLC with a model 5500 liquid chromatograph (Varian Instrument Group, Walnut Creek, CA) equipped with a UV200 variable-wavelength detector (Varian). A 100 × 4.6 mm guard column and a 250 × 4.6 mm C-18 10 μm Spherisorb column (Phase Separation, Norwalk, CT) were maintained at 30°C. The mobile phase was composed of two solvents: (A) 100% acetonitrile, and (B) 5% acetonitrile in water. The elution system involved a 30A:70B composition for 5 min followed by a linear gradient to 80A:20B over a 5 min period. The composition of the eluent was then maintained at 80A:20B for 5 min. The original column conditions were restored by a linear gradient over a 10 min time period to the composition to 30A:70B. Total run

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[2] Abbreviations: Tiron, 4,5-dihydroxy-1, 3-benzenedisulfonic acid; MSW, mycelial salt wash; LFP, lipofuscin-like pigments; SOD, superoxide dismutase; EDDHA, ethylenediaminedi-*o*-hydroxyphenylacetic acid.

time was 25 min. A constant flow rate of 1.0 ml/min was used. Elution of phenolics was monitored by absorbance at 280 nm, 0.2 *A* units/mV, with a 10.5 s time constant, and a signal-to-noise ratio of 4. An external standard method was developed with 50 to 500 ng injections of phaseollin, phaseollinisoflavan, and kievitone. The standards were generous gifts of Dr. D. A. Smith, University of Kentucky, Lexington. Retention times under these conditions were typically 10.3, 11.3, and 11.8 min for kievitone, phaseollinisoflavan, and phaseollin, respectively.

Assay of Lipid Peroxidation in Suspension-Cultured Cells. The extent of lipid peroxidation in suspension-cultured cells was determined by measuring accumulation of two lipid breakdown products, malondialdehyde (18) and lipofuscin-like pigments (LFP) (21, 30). For malondialdehyde determination, extracts of suspension-cultured cells were prepared by homogenization in 0.1% TCA and 1% SDS followed by centrifugation at 1000*g* for 15 min to remove insoluble material. Malondialdehyde was assayed in the bean extracts fluorometrically (20) by the thiobarbituric acid reaction with 1,1,3,3-tetraethoxypropane as a standard.

LFP was measured in ethanol extracts of the suspension cells prepared as described in the previous section. Lipofuscin was determined in a Gilford Fluoro IV spectrofluorometer with an excitation wavelength of 355 nm and an emission wavelength of 400 nm. Fluorescence was quantified with quinine sulfate; 1 μg/ml produced 100 units of fluorescence.

Viability of Suspension-Cultured Cells. Viability of the bean cells after various treatments was determined by the accumulation of fluorescein diacetate into the cells as described by Widholm (29). A stock solution of fluorescein diacetate (5 mg/ml) in acetone was diluted 1:10 into distilled water and then added to the suspension culture at a final concentration of 50 μg/ml. The percent of fluorescent (viable) cells was determined after 5 min with a Zeiss epifluorescence microscope with filter set 487704. Change in viability was determined from the ratio of the percent viable cells in the control and the percent viable cells in the treated cultures according to the following equation:

$$\frac{\%\ \text{Viable cells (treatment)}}{\%\ \text{Viable cells (control)}} \times 100$$

RESULTS

Elicitor activity has been previously reported for extracellular products from the α-race of *C. lindemuthianum* on bean cotyledons. These products were obtained from the mycelial surface by a salt wash (23) or from the culture filtrate (26). Purification of the culture filtrate polysaccharides, which did not adsorb to DEAE-Sephadex, produced a potent elicitor which was a galactoglucomannan (26). The crude MSW, the culture filtrate DEAE-Sephadex fraction, and the galactoglucomannan each were elicitors on both cotyledons and suspension cultured cells of the incompatible cv Dark Red Kidney (Tables I and II). After treatment of suspension-cultured cells with α-race MSW, tissue browning was observed at about 6 h, and phenolics, including the phytoalexins kievitone, phaseollin, and phaseollinisoflavan, accumulated in a time dependent manner (Fig. 1, A and B; Table II). Several separate experiments indicated that an increase in phytoalexins was first detectable in elicitor-treated compared to control cells 8 h after addition of α-race MSW (data not shown).

Treatment of suspension-cultured cells with α-race MSW elicitor also resulted in the formation of products characteristic of lipid peroxidation. Fluorescent LFP was identified by the characteristic fluorescence spectrum, which displayed excitation and emission maxima at 346 and 406 nm, respectively (Fig. 2). Phaseollinisoflavan, phaseollin, and kievitone also displayed fluorescent spectra under the conditions used to measure LFP (Fig. 2). However, the concentration (15–28 μg/g cells) of phaseollinisoflavan, which gave the most intense fluorescence, would have contributed less than 5% to the fluorescence units measured in samples from elicitor-treated cells. Accumulation of LFP in the α-race MSW-treated suspension cultured cells above control concentrations was observed between 6 and 12 h after treatment (Fig. 3A). Consequently, the timing of the production of LFP was similar to the onset of browning and accumulation of bean phytoalexins (Figs. 1B and 3A). The formation of another product of lipid peroxidation, malondialdehyde, was also observed in α-race MSW-treated cells (Fig. 3B). The use of a crude MSW preparation to demonstrate lipid peroxidation raises the possibility that this effect was a result of the activity of factors other than elicitors. Consequently, elicitor preparations from α-race culture filtrate were used at two different stages of purification to investigate the relationship between elicitor activity and LFP production. Both of these preparations, the DEAE-Sephadex fraction and galactoglucomannan, induced accumulations of LFP and enhanced phenolic synthesis (Table II).

The phytoalexin response and formation of LFP were dependent on the concentration of α-race MSW used to treat the bean suspension-cultured cells (Table III). Accumulation of LFP above controls was observed with treatment of α race MSW of 1 μg glucose equivalent/ml with a maximum effect at 10 μg glucose equivalents/ml. The α-race MSW also stimulated phytoalexin production in a concentration-dependent manner. Treatment with 1 μg glucose equivalent/ml induced phaseollin accumulation, and 5 μg glucose equivalents/ml were required for increases in kievitone and phaseollinisoflavan (Table III). Maximum responses occurred at 10 μg glucose equivalents/ml for phaseollin and at 20 μg glucose equivalents/ml for kievitone and phaseollinisoflavan.

The detection of products of lipid peroxidation upon elicitor treatment of bean suspension cells suggests involvement of activated oxygen species. Consequently, we determined whether generators of oxygen radicals would stimulate lipid peroxidation and phytoalexin production in treated bean cells. Three generator systems were used: Fe:EDDHA, Fe:EDTA, and xanthine:xanthine oxidase (4, 18, 22). Treatment of suspension-cultured cells with Fe:EDDHA and xanthine:xanthine oxidase resulted in accumulations of LFP, phenolics, and phytoalexins above control levels. The changes were similar to those observed with α-race MSW treatment (Table IV). Treatment with Fe:EDTA increased the level of LFP but no phenolic or phytoalexin accumulations were observed. Treatment with Fe:EDTA, however, caused complete loss of bean cell viability. In contrast, Fe:EDDHA or xanthine:xanthine oxidase treatments reduced cell viability to levels similar to those effected by α-race MSW (*i.e.* 49%, Table IV).

The observed necrosis of the bean suspension cells indicated the importance of monitoring viability in studies with radical scavengers or generator systems. Impaired viability of suspension-cultured cells after treatment with chemicals reported to be free radical scavengers or generators would lead to erroneous conclusions concerning the effects of those chemicals on production of LFP and phytoalexins. Two reported scavengers of hydroxyl radicals, benzoate and methionine (10), reduced bean suspension cell viability 25% and 46%, respectively (Table V). Viability was also reduced by treatment with a mixture of catalase and SOD. These enzymes should catabolize and $O_2^{\cdot-}$ or hydrogen peroxide present in the medium or plant cell surface according to the reactions

$$2H^+ + 2O_2^{\cdot-} \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2.$$

Treatments with methionine and catalase-SOD stimulated ac-

Table I. *Effect of Elicitors on Tissue Browning and the Accumulation of Phenolics and Phytoalexins in Bean Cotyledons*

Elicitor Preparation	Glucose Equivalents of Elicitor per Cotyldeon	Specific Activity[a]		Phytoalexins[b]		
		Browning	280 nm	Phaseollin	Phaseollin-isoflavan	Kievitone
	μg				*μg/g cotyledon*	
None	0	−	0	2	0	11
α Race MSW	5	+ +	26	10	11	60
Galactoglucomannan	5	+ + +	38	23	21	84
DEAE-Sephadex Polysaccharides	50	+ +	8	20	41	150

[a] Elicitor activity was determined using the cotyledon assay described by Tepper and Anderson (26). Browning is indicated by +, − indicates no browning. The α-race MSW and culture filtrate components (galactoglucomannan and DEAE-Sephadex polysaccharides) were obtained as described in "Materials and Methods." [b] Phenolics and phytoalexins were quantified as described in "Materials and Methods."

Table II. *Effect of Elicitors on Stimulation of Phenolics, Phytoalexins, and (LFP) in Bean Suspension-Cultured Cells*

Elicitor Preparation[a]	Lipofuscin[b]	Phenolics[b]	Phaseollin[b]	Phaseollinisoflavan[b]	Kievitone[b]
	% control			*μg/g cells*	
None	100	100	5	6	0
α-Race MSW	482	646	29	28	81
DEAE-Sephadex polysaccharides	954	160	22	0	1
Galactoglucomannan	490	563	36	24	110

[a] Dark Red Kidney bean suspension-cultured cells (2 g/50 ml) were treated at 22°C for 24 h with elicitors: α-race MSW 10 μg glucose equivalents/ml, galactoglucomannan 10 μg glucose equivalents/ml, and partially purified polysaccharides 20 μg glucose equivalents/ml. The α-race MSW and culture filtrate components (galactoglucomannan, and polysaccharides) were obtained as described in "Materials and Methods." [b] LFP, phenolics, and phytoalexins were quantified as described in "Materials and Methods." Control values were: LFP, 1.5×10^3 fluorescent units/g cells; phenolics, 187 μg salicylic acid equivalents/g cells.

cumulations of LFP, phenolics, and phytoalexins (Table V). Because benzoate alone did not appreciably stimulate LFP or phenolic accumulation (Table V), the effect of this putative scavenger upon the response of bean cells to α-race MSW-treatment was examined. Although addition of benzoate to MSW treated cells reduced the levels of LFP, phenolics, and phytoalexins, complete necrosis of the bean cells was observed with this combined treatment (Table V).

Bean cell viability was not reduced by treatment with Tiron, a superoxide anion quencher (16), and formation of LFP, phenolics, or phytoalexins was not enhanced (Table V). Co-treatment of cells with Tiron and free radical generators, Fe:EDDHA or xanthine-xanthine oxidase, increased viability and reduced accumulations of LFP, phenolics, and phytoalexins relative to cells treated with the generators alone (Table IV). However, in co-treatments with α-race MSW, although Tiron did reduce the extent of necrosis and the production of phytoalexins, more LFP and phenolics accumulated than in cells treated with α-race MSW alone (Table V).

Treatment of bean cells with mannitol, another hydroxyl radical scavenger, did not alter viability or stimulate accumulation of LFP, phenolics, or phytoalexins. In studies of treatments with both α-race MSW and mannitol, some experiments (*e.g.*, Expt. 1, Table V) displayed no protective effect as shown by enhanced LFP and phenolic levels. In other studies LFP and phenolic accumulations were reduced relative to α-race MSW treatments (*e.g.* Expt. 2, Table V). The reason for this variability is unknown. Different batches of suspension cultured cells also varied in the extent of response to elicitor. Treatments with α-race MSW at 10 μg glucose equivalents/ml resulted in values for LFP from 310 to 517% of controls and accumulation of phenolics from 524 to 646% of controls. Variability in phytoalexin production ranged from 14 to 29 μg/g cells for phaseollin, 5 to 28 μg/g cells for phaseollinisoflavan, and 25 to 73 μg/g cells for kievitone (Tables II and III). Consequently, although the data for each experiment are representative of at least three replications, the data provided in each table were collected from one cell batch for all treatments in that table.

DISCUSSION

Extracellular products from the α-race of the bean pathogen *C. lindemuthianum* stimulated phenolic production in cotyledons and suspension cells of an incompatible cv Dark Red Kidney. Treatment of suspension-cultured cells with crude α-race MSW preparations and galactoglucomannan induced the synthesis of kievitone and, to a lesser extent, phaseollin and phaseollinisoflavan.

Although α-race MSW preparations consistently induced the accumulation of phenolics and phytoalexins above control values, the intensity of the response was variable between cell batches. Hamdan and Dixon (12) report similar variability in elicitor responses between batches of suspension cultured cells of bean cultivars.

Products indicative of lipid peroxidation, lipofuscin and malondialdehyde, accumulated coincidently with phenolics and phytoalexins in suspension cells treated with either crude mycelial salt wash or purified culture-filtrate elicitors. Products of lipid peroxidation with fluorescence spectra similar to the LFP extracted from elicitor-treated suspension cells have been previously reported. The excitation and emission maxima that we observe (346/406 nm) are lower than those reported for lipofuscin in animal tissues (360/430–470 nm) (6) or senescent bean cotyledons (370/430 nm) (21). However, similar values (350/408 nm) have been documented for LFP in chloroplasts from senescent bean leaf tissue (30).

Induced accumulations of LFP, phenolics, and phytoalexins also occurred when bean cells were treated with generators of activated oxygen species. Thus, these generators display elicitor-like activity. The xanthine:xanthine oxidase system is documented to produce the superoxide anion $O_2^{\cdot-}$ (4). Chelates of iron are reported (17, 22) to produce the hydroxyl radical $OH^{\cdot}$ through the following reaction:

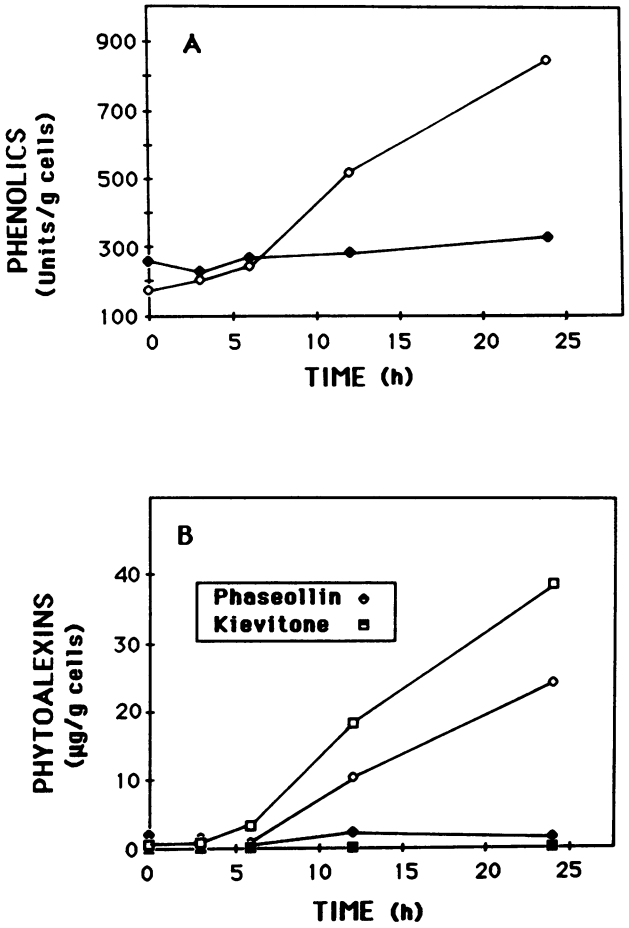

FIG. 1. Production of phenolics and phytoalexins, phaseollin, and kievitone in bean suspension-cultured cells, treated with α-race MSW. Bean suspension-cultured cells were incubated in the presence (○, □) or absence (●, ■) of α-race MSW (10 µg glucose equivalents/ml). A, Cells were extracted and phenolics determined as described in "Materials and Methods." One unit of phenolic activity represents 1 µg salicylic acid equivalent measured at 280 nm (26). B, Cells were extracted and phaseollin (○, ●) or kievitone (□, ■) determined by HPLC, as described in "Materials and Methods.

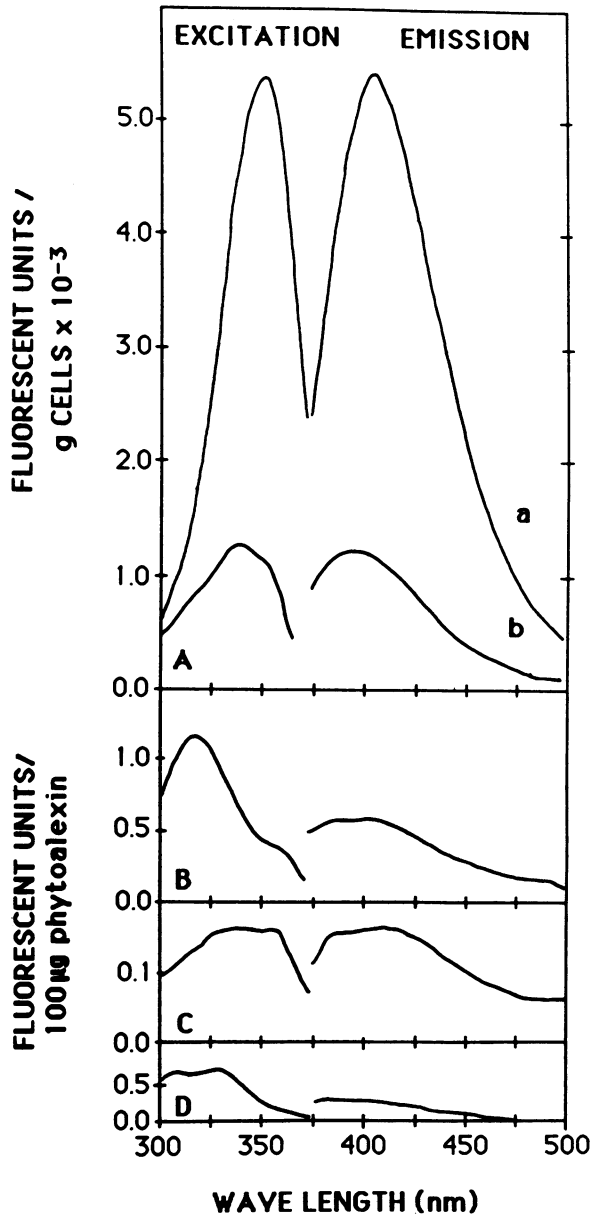

FIG. 2. Fluorescence spectra of (A) ethanol extracts from bean suspension-cultured cells incubated for 24 h in the presence (a) or absence (b) of α-race MSW (10 µg glucose equivalents/ml); (B) phaseollinisoflavan, (C) kievitone, (D) phaseollin. Cells were extracted for lipofuscin-like pigments as described in "Materials and Methods." Phytoalexin standards were prepared in ethanol as described in "Materials and Methods"; fluorescence spectra represent the intensities for 100 µg of each compound. For all excitation spectra λ_{em} = 406 nm; for all emission spectra λ_{ex} = 346 nm.

$$Fe^{2+} + H_2O_2 \longrightarrow OH^{\cdot} + Fe^{3+} + OH^{-}.$$

This reaction would be perpetuated by systems that reduce Fe^{3+} and provide H_2O_2. The H_2O_2 may be supplied by oxidation of NAD(P)H by wall-associated peroxidase or by dismutation of superoxide anion by SOD. Mechanisms that may reduce iron in the bean cells include the NADPH-linked electron transport systems reported by Lin (15), Bienfait (2), and Barr *et al.* (1) or by ascorbate, a known plant cell metabolite (19).

The behavior of the activated-oxygen generating systems as elicitors suggests that the fungal components with elicitor function may stimulate free radical formation in the plant cells. Previous studies with free radical scavengers suggest that the induction of phytoalexins by $AgNO_3$ involves the hydroxyl radical $OH^{\cdot}$ (10). Doke (8) demonstrated production of $O_2^{\cdot-}$ by a NAD(P)H-dependent process in potato cells to be stimulated by elicitors from *Phytophthora infestans*.

The expected inhibition by free radical scavengers of the effects of fungal elicitors in bean cells was not confirmed. One explanation may be that elicitors are so effective in causing free radical production that once the normal scavenging mechanisms of the plant cell are exceeded even additional external scavengers are ineffective. However, the results with benzoate and methionine as free radical scavengers may be confounded because they produced necrosis of the bean cells. Indeed, methionine and SOD:catalase acted like elicitors in causing LFP, phenolics, and phytoalexins to accumulate in suspension-cultured cells. The effects of methionine may be related to a light-mediated reaction between methionine and isoflavan provided in the culture medium (7) to produce free radicals (24). It has also been shown

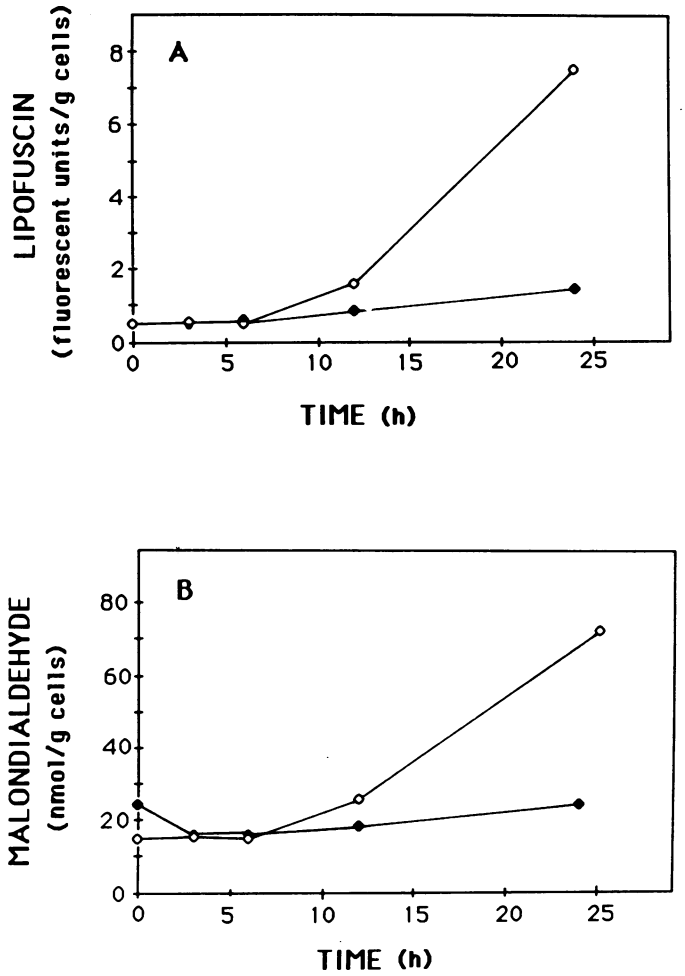

FIG. 3. Formation of products characteristic of lipid peroxidation as a result of treatment of bean suspension cultured cells with α-race MSW. Bean suspension-cultured cells were incubated in the presence (○) or absence (●) of α-race MSW (10 μg glucose equivalents/ml). A, Cells were extracted and LFP determined as described in "Materials and Methods." Fluorescence was quantified with quinine sulfate; 1 g/ml produced 100 fluorescent units. B, Cells were extracted and malondialdehyde was determined as described in "Materials and Methods."

that catalase may stimulate lipid peroxidation (27). Another unexpected observation was the stimulation of cell death caused by treatment with both benzoate and α-race MSW. This effect may reflect on the ability of benzoate to accelerate the rate of lipid perioxidation, as observed when lipid peroxidation was triggered by a $Fe\text{-}H_2O_2$ system (18). Our variable results with mannitol as a protectant for α-race MSW treatment are similar to reports in which mannitol accelerated lipid peroxidation in some systems but inhibited lipid peroxidation in others (18). Minotti and Aust (18) suggest that the effect of mannitol is related to the Fe^{2+}/Fe^{3+} ratio, which in plant cells may vary with metabolic state. Although Tiron failed to protect against α-race MSW treatments, partial protection was observed with the free radical generator systems. The putative elicitor-generated free radicals may be inaccessible to Tiron, or the mechanism may not be sensitive to removal of $O_2^{\cdot-}$. This latter scenario may occur if lipid peroxidation results from lipohydroperoxides generated by lipoxygenase activity, *i.e.*

$$\underset{LH}{Lipid} + O_2 \xrightarrow{\text{lipoxygenase}} \underset{LOOH}{\text{lipohydroperoxide}}$$

$$LOOH \xrightarrow{M^+} LO^{\cdot} + OH^-$$

$$LH + LO^{\cdot} \longrightarrow L^{\cdot} + LOH$$

Thompson and colleagues (16, 17) have associated $O_2^{\cdot-}$ production with lipoxygenase activity in senescing bean tissues. Thus, Tiron may be expected to enhance rather than inhibit lipohydroperoxide formation because of its ability to quench the $O_2^{\cdot-}$ generated from a lipoxygenase-catalyzed reaction. A role of lipoxygenase has been proposed in hypersensitivity incited by incompatible bacteria in cucumber (14) and incompatible viruses in cowpea (13) as well as in response of potato to elicitors from *P. infestans* (25).

The observation that an elicitor causes lipid peroxidation is significant in several ways. The alteration in lattice structure of membranes due to lipid peroxidation may be involved in changes in plasmalemma permeability, which is a characteristic of elicitor treatments and hypersensitivity (28). Indeed, peroxidation induced by ozone treatment reduced the activity of the K^+-stimulated ATPase in pinto bean (9). Impaired membrane structure and function may eventually contribute to plant cell necrosis observed in hypersensitivity. The possible presence of such free radicals as $OH^{\cdot}$, $O_2^{\cdot-}$, and lipid radicals or lipohydroperoxides in reacting plant cells extends the types of antimicrobial chemicals that may be used in defense. These molecules would be deleterious to the structure and function of enzymes and surface components of challenging microorganisms (11). These active oxygen species may also act as triggers for the plant cells to alter cellular metabolism, a concept that has been proposed for bacterial cells (3). Perhaps "alarmone" type nucleotides (3) are produced in plant cells in response to elicitors. Such alarmones may participate in triggering enhanced expression of genes concerned with production of phenolics and other metabolites that change upon elicitor treatment (7).

Table III. *Effect of α-Race MSW Concentration on the Accumulation of (LFP), Phytoalexins, and Phenolics in Treated Bean Suspension-Cultured Cells*

Treatment[a]	LFP[b]	Phenolics[b]	Phaseollin[b]	Phaseollinisoflavin[b]	Kievitone[b]
	% control			*μg/g cells*	
Control	100	100	<1	<1	<1
α-Race MSW (μg/ml):					
0.1	107	102	<1	<1	<1
1.0	150	131	1	<1	<1
5.0	352	270	10	1	5
10.0	517	614	14	8	25
20.0	330	558	10	9	32

[a] Dark Red Kidney suspension-cultured cells (1–2 g) were treated at 22°C for 24 h with α-race MSW at concentrations of 0 to 20 μg glucose equivalents/ml. The α-race MSW was obtained as described in "Materials and Methods." [b] LFP, phytoalexins, and phenolics were measured as described in "Materials and Methods." Control values were: LFP, 0.40 × 10^3 fluorescent units/g cells; phenolics 91 μg salicylic acid equivalents/g cells.

Table IV. *Effect of Active Oxygen 'Generators' on Suspension-Cultured Cell Viability and the Accumulations of LFP, Phenolics, and Phytoalexins*

Treatment[a]	Percent of Control[b]			Phytoalexins[b]		
	Viability	LFP	Phenolics	Phaseollin	Phaseollin-isolfavin	Kievitone
					μg/g cells	
Control	100	100	100	7	0	1
α-Race MSW	48	310	524	19	5	73
Fe:EDDHA	49	1000	697	33	4	22
Fe:EDDHA + Tiron	75	620	388	21	3	8
Xanthine:xanthine oxidase	49	940	424	39	7	28
Xanthine:xanthine oxidase + Tiron	90	600	348	22	4	15
Fe:EDTA	0	1000	109	5	0	3

[a] Dark Red Kidney bean suspension-cultured cells (2 g/50 ml) were treated at 22°C for 24 h with: α-race MSW 10 μg glucose equivalents/ml; Fe:EDDHA 5 mM; Tiron, 1 mM; xanthine, 1 mM; xanthine oxidase, 18 μg/ml; and $FeSO_4$, 5 mM; EDTA, 5 mM. [b] Viability, LFP, phenolics, and phytoalexins were quantified as described in "Materials and Methods". Control values were viability, 83%; LFP, 1.0×10^3 fluorescent units/g cells; phenolics, 33 μg salicylic acid equivalents/g cells.

Table V. *Effect of Active Oxygen 'Scavengers' on Bean Suspension-Cultured Cell Viability and Accumulation of LFP, Phenolics, and Phytoalexins in MSW Treated and Nontreated Cells*

Treatment[a]	Percent of Control[b]			Phytoalexins[b]		
	Viability	LFP	Phenolics	Phaseollin	Phaseollin-isoflavin	Kievitone
					μg/g cells	
Control	100	100	100	0	0	0
Mannitol Expt. 1	110	110	72	2	0	0
Mannitol Expt. 2	ND[c]	125	81	ND	ND	ND
Tiron	100	36	47	2	0	0
Benzoate	75	94	152	0	0	0
Methionine	54	242	296	43	3	11
SOD + catalase	50	1182	435	45	0	13
α-Race MSW	48	310	524	19	5	73
MSW + mannitol Expt. 1	47	390	830	20	7	12
MSW + mannitol Expt. 2	ND	108	98	ND	ND	ND
MSW + Tiron	55	510	697	7	17	17
MSW + benzoate	0	147	228	1	0	0

[a] Dark Red Kidney bean suspension-cultured cells (2 g/50 ml) were treated at 22°C for 24 h with: α-race MSW 10 μg glucose equivalents/ml; mannitol 50 mM; Tiron, 1 mM; sodium benzoate 5 mM; methionine 50 mM; or SOD, 32 μg/ml; and catalase, 40 μg/ml. The α-race MSW was obtained as described in "Materials and Methods." [b] Viability, LFP, phenolics, and phytoalexins were quantified as described in "Materials and Methods." Control values were: viability 83%; LFP 1.0×10^3 fluorescent units/g cells; phenolics 33 μg salicylic acid equivalents/g cells. [c] Not determined.

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