Rapid Stimulation of 5-Lipoxygenase Activity in Potato by the Fungal Elicitor Arachidonic Acid

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ABSTRACT

The activity of lipoxygenase (LOX) in aged potato tuber discs increased by almost 2-fold following treatment of the discs with the fungal elicitor arachidonic acid (AA). Enzyme activity increased above that in untreated discs within 30 min after AA treatment, peaked at 1 to 3 h, and returned to near control levels by 6 h. The majority of the activity was detected in a soluble fraction (105,000g supernatant), but a minor portion was also associated with a particulate fraction enriched in microsomal membranes (105,000g pellet); both activities were similarly induced. 5-Hydroperoxyeicosatetraenoic acid was the principal product following incubation of these extracts with AA. Antibodies to soybean LOX strongly reacted with a protein with a molecular mass of approximately 95-kD present in both soluble and particulate fractions whose abundance generally corresponded with LOX activity in extracts. LOX activity was not enhanced by treatment of the discs with nonelicitor fatty acids or by branched β-glucans from the mycelium of Phytophthora infestans. Prior treatment of the discs with abscisic acid, salicylhydroxamic acid, or n-propyl gallate, all of which have been shown to suppress AA induction of the hypersensitive response, inhibited the AA-induced increment in LOX activity. Cycloheximide pretreatment, which abolishes AA elicitor activity for other responses such as phytoalexin induction, did not inhibit LOX activity in water- or elicitor-treated discs but enhanced activity similar to that observed by AA treatment. The elicitor-induced increase in 5-LOX activity preceded or temporally paralleled the induction of other studied responses to AA, including the accumulation of mRNAs for 3-hydroxy-3-methylglutaryl coenzyme A reductase and phenylalanine ammonia lyase reported here. The results are discussed in relation to the proposed role of the 5-LOX in signal-response coupling of arachidonate elicitation of the hypersensitive response.

During the past several years, considerable progress has been made in the molecular characterization of plant LOXs

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3 Abbreviations: LOX, lipoxygenase; AA, arachidonic acid; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, (linoleate:oxygen oxidoreductase, EC 1.13.11.12), and several LOXs from different species now have been cloned and characterized (reviewed in ref. 34). However, the defined functions of LOXs in physiological and metabolic processes in plants remain poorly understood. These enzymatic reactions are catalyzed by fatty acids containing a cis,cis,1,4-pentadiene configuration in their corresponding hydroperoxides (40). Plants have different LOX isoforms with different catalytic specificities, but the factors that regulate the expression of these isoforms are largely unknown (12, 18, 34). Various roles have been proposed for LOXs that include a role in plant growth and development, senescence, and defense against insects and pathogens (7, 17, 18, 28, 34), although definitive proof for any of these is lacking. The normal substrates for plant LOXs are linoleic and linolenic acids, and LOX products of these fatty acids are metabolized to compounds that may function as signal molecules. These include methyl jasmonate, involved in pestinase inhibitor induction (11), and traumatin (40). During fungal infection, the intrusion of fatty acids not commonly found in higher plants, such as the elicitors AA and EPA present in Phytophthora spp. (5, 7), presents plant LOXs with unusual substrates to produce other highly reactive metabolites that may participate in the induction of defense-related processes (7, 27).

LOXs can be classified as 5-, 12-, or 15-LOXs, etc., according to where in the carbon chain of AA the hydroperoxy function is introduced. The 5-LOX, the principal LOX activity in Solanum tuberosum (12, 23, 29, 33), has been proposed to be an intermediate enzyme in the pathway between treatment with AA and the altered metabolism and necrosis associated with the HR (5, 28). The compelling evidence for this are the structural features in fatty acid elicitors (i.e. AA, EPA, and related compounds) that are required for elicitor activity, all of which meet the substrate requirements for the 5-LOX (5, 27): the abolition of AA elicitor activity by LOX inhibitors (28); and recent studies by Vaughan and Lulai (39), who reported that, unlike its strong elicitor activity in potato calli containing normal LOX activity, AA did not elicit responses in LOX-null calli.

Despite progress in the purification of the 5-LOX, little work has been reported concerning the spatial or temporal distributions of LOX activity and the hydroperoxyeicosatetraenoic acid; HPODE, hydroperoxyoctadecadienoic acid; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; HR, hypersensitive response; PAL, phenylalanine ammonia lyase; PG, n-propyl gallate; SHAM, salicylhydroxamic acid.
response of this enzyme following exposure of potato plants to pathogenic agents or their elicitors (4, 12). Galliard and colleagues (reviewed in ref. 12) provided the initial biochemical characterization of LOX activity in potato and provided evidence for the association of enzyme activity in tuber protoplasts with labile, lysosome-like particles. As a prelude to more detailed studies of 5-LOX expression in defense-related responses, we examined 5-LOX activity following treatment of potato discs with AA and with compounds known to affect AA elicitor activity. In this paper, we report the specific, rapid, and transient enhancement of 5-LOX activity following AA treatment of tuber discs, a response that precedes the activation of other elicitor-induced responses in this system.

MATERIALS AND METHODS

Plant Material and Experimental Treatments

Certified seed potatoes (Solanum tuberosum L. cv Kennebec) were obtained from commercial sources and used throughout this study. Conditions for storage of the tubers, preparation and treatment of tuber discs with AA, and extraction and analysis of sesquiterpenoid phytoalexins were as described previously (5, 6). After preparation, discs were aged in the dark at 20°C for 16 to 20 h before application of treatment solutions. Purified HPEtEs (see below) were applied as 5% (v/v) ethanolic solutions. Inhibitor solutions were applied and spread over the upper surface of each disc (50 μL of stock solution per disc), and the discs were incubated for 3 h in the dark at 20°C before treatment with AA or water. After treatment with AA (3.3 mm aqueous emulsion; 0.17 μmol per disc) or water, applied in the same fashion to the same surface as the inhibitor, the discs were incubated in the dark at 20°C until extraction. This is a concentration of AA that is 50 to 75% maximal with respect to elicitation of sesquiterpene phytoalexin accumulation (5, 6). PG was applied as a 10-mm aqueous stock solution, ABA as a 7.6-mm solution in 5% methanol, and SHAM as a 10-mm solution in 50 mm Mes, pH 5.5 (28). Control solutions for these treatments were water, 5% methanol, or Mes buffer. For the experiments with cycloheximide, aged discs were immersed with shaking (150 rpm) at room temperature for 2 h in a solution containing 0.3 mM mannitol, 40 mM Mes, pH 5.8, with or without 100 μM cycloheximide (40 discs per 100 mL). Treatment with cycloheximide under these conditions nearly completely inhibited protein synthesis (98% inhibition) as determined by the method described by Stermer and Bostock (37). The discs were then placed in Petri dishes, and water or AA (100 μg per disc) was applied to the upper surface. The discs were extracted 2 h later and assayed for AA activity by the polarographic method described below.

Chemicals

All fatty acids were purchased from Sigma Chemical Co. (St. Louis, MO) and were of the highest purity (>99%). SHAM, PG, (+/-)-ABA, and cycloheximide were also from Sigma. [1-14C]AA (40–60 mCi mm−1) was from New England Nuclear. Soybean lipoxidase, type I, was from Sigma (specific activity 112,000 units mg−1). Purified 5-HPETE, 15-HPETE, 9-HPEDE, and HETE standards were from Cayman Chemical Co. (Ann Arbor, MI).

Extraction of Tissues and Preparation of Microsomes

LOX activity was extracted from the top 1 mm of the potato discs. The upper mm of 8 to 10 discs (approximately 3 to 4 g fresh weight of tissue) was homogenized with a Polytron homogenizer (speed 5 for 1 min; PT-10 probe; Brinkmann) in 15 to 20 mL of cold buffer containing 0.2 M potassium phosphate buffer (pH 7.5), 0.35 M sorbitol, 5 mM MgCl2, 10 mM EDTA, 5% polyvinylpyrrolidine, 20 mM β-mercaptoethanol, 0.7 μg mL−1 of pepstatin, 0.5 μg mL−1 of leupeptin, and 0.5 mM PMSF. The extract was filtered through two layers of cheesecloth into a 50-mL centrifuge tube. The filtered homogenate was centrifuged at 12000g for 5 min and then at 16,000g for 30 min. The 16,000g supernatant, after dilution with glycerol (20% final concentration), was assayed for LOX activity or, to obtain microsomes, was centrifuged without glycerol addition at 105,000g for 60 min. All steps were performed at 4°C. The 105,000 g pellet (i.e. the microsomal fraction) was resuspended overnight on ice in 200 μL of cold buffer containing 0.1 M potassium phosphate (pH 7.5), 20% glycerol, 0.1 mM DTT, 1 mM EDTA, and 0.4 mM PMSF. Any residual microsomal pellet was suspended in the buffer by gentle pipetting. This typically provided a microsomal concentration of 3.7 to 4.5 mg of protein mL−1. Protein content was determined by the Bio-Rad dye-binding method following the manufacturer’s directions with ovalbumin or BSA as the standard.

Enzyme Assays

The LOX activity in the soluble fraction was measured polarographically with an O2 electrode (2; Yellow Springs Instruments, Yellow Springs, OH). Lipoxygenase activity in microsomes was measured spectrophotometrically (A236) by the increase in conjugated diene formation with cis-9,12-linoleic acid as substrate (2). Enzyme assays were conducted at 25°C. For polarographic measurements, the reaction mixture contained 350 μM linoleic acid, Triton X-100 (0.01%), 0.1 mM acetate buffer (pH 5.5), and 100 μL of the extract in a total volume of 1.7 mL. For the spectrophotometric assay, the reaction mixture included 75 μM linoleic acid, 0.005% TWEEN 20, 0.1 mM acetate buffer (pH 5.5), and 10 μL of resuspended microsomes in a total volume of 1.2 mL (23). One unit of LOX activity is defined as 1 μmol HPODE produced per min (ε = 25,000 M−1 cm−1; ref. 23). Usually, two determinations were made for each sample within each experiment. The majority of the LOX activity (approximately 99%) was present in the 105,000g supernatant. Therefore, unless indicated otherwise, the enzyme activity in the 16,000g supernatant (henceforth soluble fraction) was measured in most experiments. The 105,000g supernatant and pellet fractions were also compared for the microsomal enzyme activities: antimycin A-insensitive NADH Cyt c reductase and NADPH Cyt c reductase (13). Our microsome preparations were highly enriched for these activities. The specific activities for these enzymes in potato microsomes were, respectively, 421 and 132 nmol Cyt c reduced min−1 mg−1 of protein. The
corresponding values for the 105,000g supernatant were 51 and 33 nmol Cyt c reduced min⁻¹ mg⁻¹ of protein. Reaction conditions were as described before (13).

**Extraction and Analysis of AA Metabolites**

To obtain LOX products of AA, 10 μL of the microsomal suspension were added to 600 μL of 0.2 M sodium acetate buffer (pH 5.5), 530 μL of H₂O, and 60 μL of 1.5 mm AA containing [¹⁴C]AA. After 3 min, the mixture was transferred to 1 mL of boiling isopropanol (80°C) and extracted with chloroform:methanol (2:1, v/v), and nonlipid contaminants were removed with 0.75% KCl. Lipid extracts were dried under N₂, resuspended in 1 mL of ethanol, and reduced by addition of 40 μL of 100 mm NaBH₄, (Fig. 1) to convert HPETEs to their more stable HETEs. Reduced extracts were acidified with 40 μL of acetic acid, diluted to 25% ethanol, and then enriched for HETEs by chromatography on Sep-Pak ODS-silica cartridges (Waters Associates, Milford, MA, ref. 26). The HETE fraction, eluted from the cartridge with petroleum ether:chloroform (65:35, v/v) and then concentrated under a stream of N₂, was analyzed by HPLC with UV and radioisotope detectors (Beckman Instruments, Fullerton, CA). HETEs were separated on a 10 μM silica column (Microporasil, 3.9 mm × 30 cm; Waters Associates) in a solvent mixture of hexane:ethanol:acetic acid (983:16:1, v/v/v; ref. 36). The initial flow rate of the mobile phase was 0.8 mL min⁻¹ and changed to 1.5 mL min⁻¹ after 20 min. Column effluent was monitored for UV A₂₃₄ and for radioactivity. To measure radioactivity, the effluent was mixed in line with liquid scintillation fluid (Scintiverse, Fisher) in a 1:3 ratio (effluent:scintillation fluid), and the flow cell volume of the detector was adjusted to 1 mL. For peak identification and quantitation, retention times of individual HETE standards were established.

**Immunodetection of LOX in Potato Extracts**

Components in potato extracts (10–20 μg of protein per lane) were separated by SDS-PAGE in 10 or 12.5%, 0.75-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Peroxidation of AA by 5-LOX to 5-HPETE, an intermediate in the pathway leading to leukotrienes and other eicosanoids. HPETEs were reduced with NaBH₄ to their corresponding HETEs before HPLC analysis.

**Gel Blot Analyses for HMGR and PAL mRNA Accumulation**

Total RNA was prepared from potato tuber discs by the method described by Harada et al. (14). Total RNA (20 μg per lane) was fractionated by electrophoresis through 1% agarose gels containing formaldehyde and transferred to Nytran membranes (Schleicher and Schuell). Hybridization of cDNA probes to RNA blots routinely was carried out in 50% formamide, 1 M Na⁺, 42°C, and washed at the same criterion by standard procedures (1). The amount of labeled probe hybridized to each RNA sample was estimated with a two-dimensional radiosotope imaging system (Ambis, Inc., San Diego, CA).

The DNA probe used to determine HMGR mRNA abundance was a 360-bp EcoRI-AsuII fragment from pHMG3 corresponding to a portion of the 5′ region of the potato *hmgs*3 gene (8). The DNA probe used for determining PAL mRNA abundance was a 780-bp EcoRI fragment from *pTPAL1*-1.11, which contains the *pal1* gene from tomato (*Lycopersicon esculentum* L.; gift of L. Bloksberg and C.I. Kado, ref. 3). The probe corresponds to a region that is conserved among PAL genes.

**RESULTS**

**Induction of 5-LOX Activity**

Treatment of tuber discs with AA rapidly increased the LOX activities associated with both the soluble and the microsomal fractions. The activities increased by as much as 1.75-fold over the nontreated control after elicitor treatment.
Figure 2. Time course of induction of LOX activities in soluble and particulate fractions from tuber discs extracted after treatment with AA (0.17 μmol per disc). Values are expressed relative to those from the corresponding fractions in water-treated control discs and are the mean ± st from five separate experiments (soluble, □) or two experiments (microsomal, ■). LOX activities in extracts of water-treated discs did not change significantly during the sampling period and were 0.123 ± 0.002 units/mg of protein (microsomal) and 0.336 ± 0.022 units/mg of protein (soluble).

(Fig. 2). The increase was apparent by 30 min after treatment, peaked at 1 to 3 h, and then declined to near control levels. Incubation of microsomes with [14C]AA and analysis of the HPETEs produced by the reaction (measured as the corresponding HETEs) indicated that 5-HPETE is a major product of this activity (Fig. 3). A similar profile of HPETEs products was observed after incubation of AA with the 16,000g supernatant (data not shown). This confirmed that 5-LOX activity is indeed associated with both preparations. Other major products that were detected were 8-, 9-, 11-, 12-, and 15-HPETE, consistent with the spectrum of hydroperoxides reported by Redanna et al. (29). The pH optimum for the microsomal 5-LOX activity was 5.5, identical with that reported for potato LOX preparations by Galliard (12) and more recently with highly purified preparations by Mulliez et al. (23). The curve for the time course of AA induction of microsomal LOX activity was identical whether linoleic acid or AA was used as the assay substrate. However, the activity with linoleic acid, an endogenous substrate, was higher than with AA, also consistent with results reported by Mulliez et al. (23) using the purified enzyme.

Immunodetection of Potato LOX

The soybean LOX antibodies reacted strongly with an abundant 95-kD protein and with a less-abundant protein of lower molecular mass (approximately 88 kD; Fig. 4). At this degree of resolution, the 95-kD protein had a mobility within the gels almost identical with the predominant, immunoreactive components in the soybean LOX preparation. The size of the principal immunoreactive component compares with the reported molecular mass of 95 kD of the major potato tuber LOX isozyme reported by Shimizu et al. (33) and with other estimates ranging from 85 to 100 kD reported by other investigators using various methods (12, 23, 29). Proteins of the same mass reacted with the antibody in the microsomal preparations (Fig. 4B), but these were present as relatively minor components in the samples. One major and several minor bands in the microsomal extracts corresponding to components <11 kD cross-reacted with the antibody (Fig. 4B, lane 1), but their abundance varied considerably in preparations and may represent degradation products.

Two other components (35 and 31 kD) reacted weakly with the antibody in immunoblots of extracts following SDS-PAGE. One of these of approximately 35 kD may correspond to the L2 isozyme, which, although catalytically active, appears to be a breakdown product of the 95-kD protein (29). The relative abundance of the 95- and 88-kD components appeared to correspond with the LOX activity in extracts, although more precise quantitative methods such as ELISA to establish this correlation were not attempted in our study.

Figure 3. HPLC profile of reduced products from reaction of [14C]-AA with a microsomal LOX preparation. HPETEs were reduced to their corresponding HETEs with NaBH₄. HETEs were separated on a 10-μm Microporasil column (3.9 mm × 30 cm) with hexane:ethanol:acetic acid (983:16:1, v/v/v) as the mobile phase as described in "Materials and Methods."
These proteins also were clearly apparent after treatment of blots with colloidal gold (not shown). Our results corroborate unpublished data of D. Hildebrand (personal communication), who has also observed that this antibody preparation cross-reacts with LOXs in potato and tobacco.

**Specificity of Inducers**

To determine the specificity of the microsomal LOX response, nonelicitor fatty acids were applied to potato discs and then the discs were extracted 3 h later. Neither cis-9,12-linoleic acid nor arachidic acid enhanced LOX activity (Fig. 5). β-Glucans purified from *P. infestans* mycelium and containing predominantly 3-, 6-, and 3,6-linked glucosyl residues strongly enhance the phytoalexin elicitor activity of AA and EPA (22). To determine whether this effect was evident at the level of 5-LOX activity, we added glucans (1 mg glucose equivalents per mL) to the AA treatment solution and then determined the LOX activity 3 h after treatment. The glucan alone had no effect on LOX activity but suppressed the AA-induced increase by 77% (not shown).

**Effects of Inhibitors of AA Elicitor Activity on 5-LOX Induction**

ABA, PG, and SHAM inhibited the AA-induced increase in microsomal LOX activity (Fig. 6). All three were about equally effective at the concentrations tested and inhibited LOX activity by 40 to 46% relative to the AA-induced control. The degree of inhibition by PG and ABA is comparable to their effect on AA-induced sesquiterpenoid phytoalexin accumulation observed in previous studies using these same concentrations (7). SHAM nearly completely suppresses phytoalexin accumulation under comparable conditions (28). These inhibitors nearly abolished the AA-induced increment in LOX activity.

In contrast to the other inhibitors, cycloheximide did not inhibit LOX activity. Cycloheximide completely blocks AA induction of sesquiterpenoid phytoalexins and other biochemical responses normally induced by this elicitor (our unpublished observations). Surprisingly, cycloheximide en-
hanced LOX activity (Fig. 7), similar to that observed following AA treatment of the discs. In immunoblots, the 88- and 95-kD components were as abundant in extracts of cycloheximide-treated discs as in the controls (Fig. 7B).

**Effect of Ca\(^{2+}\) on Microsomal and Soluble LOX Activities**

Because Ca\(^{2+}\) ion has been reported to enhance AA-induced accumulation of sesquiterpenoid phytoalexins (42) and to stimulate the mammalian 5-LOX activity (20), we tested CaCl\(_2\) for its possible effect on LOX activity in our preparations. CaCl\(_2\) caused a modest stimulation of microsomal LOX activity when microsomes were incubated in the reaction buffer for 15 min at room temperature before addition of the substrate. The effect was evident between Ca\(^{2+}\) concentrations of 100 \(\mu\)M and 2 mM and was greatest at 1 mM (by 14%). However, a similar effect on the soluble activity was not observed, consistent with the observations of Mulliez et al. (23), who found that the activity of purified preparations of the major potato LOX activity were unresponsive to Ca\(^{2+}\).

**LOX Induction in Relation to HMGR and PAL mRNA Accumulation**

The AA-induced increase in 5-LOX activity preceded or temporally paralleled the AA-induced increases in mRNA levels for PAL and elicitor-responsive isoforms of HMGR. The increase in PAL mRNA was detected within 1 h after AA treatment, and the increase in HMGR mRNA was detected within 2 h after treatment, with transcript abundance for both mRNAs attaining a maximum level by 4 h (Fig. 8).

**Effect of HPETEs on Sesquiterpenoid Phytoalexin Accumulation**

In one of three trials, 5-HPETE at 10 \(\mu\)g (0.03 \(\mu\)mol) per disc elicited low levels of sesquiterpenoid phytoalexins (32 pg of rishitin plus lubimin per g fresh weight). The elicitor activity was inconsistent, and lower concentrations of 5-HPETE were inactive. AA at this concentration elicited 48 \(\mu\)g of rishitin and lubimin per g fresh weight. 15-HPOTE and 9-HPODE at 10 \(\mu\)g per disc were inactive in all experiments. The inconclusive results with 5-HPETE seem contradictory with other biochemical evidence, which indirectly suggest its participation in elicitation. However, our observations might be explained by the instability of 5-HPETE and the abundance of wall-bound peroxidases in potato discs (6) that could reduce exogenously applied 5-HPETE to 5-HETE and, thereby, prevent its interaction with cellular sites of action.

**DISCUSSION**

The results of our study indicate that the enhancement of LOX activity by AA is a specific and rapid response to this elicitor. This induction precedes other responses studied in earlier investigations (5–7, 27, 37) and precedes or temporally parallels the increase in abundance of transcripts for PAL and HMGR that occur 1 to 2 h after elicitor treatment of aged discs. PAL and HMGR induction is essential for the biosynthesis of lignin and sesquiterpenoid phytoalexins, respectively, which accumulate during expression of resistance to *P. infestans* in this system (6, 7). LOX induction following AA treatment and the absence or only modest response to non-elicitor fatty acids, including the LOX substrate linoleic acid, suggests that the stress imposed by AA to the tissue involves generation of specific signals to induce the response. Such specificity by AA and related fatty acids is also observed for elicitor effects on HMGR gene expression (8) and the sesquiterpenoid phytoalexin response in this system (5, 27).

At the level of resolution of our study, we did not see evidence for differential activation of LOX isoforms. Only a small fraction of the total activity recovered in extracts was present in the microsomal fraction (<1%), and the soybean LOX antibody recognized similar size proteins in immunoblots of soluble (105,000g supernatant) and microsomal (105,000g pellet) fractions. Because the microsomal activity represented such a small fraction of the total, we did not characterize it further, and we believe that most of the activity of our preparations likely derives from the same enzyme(s) as in the soluble fraction. The LOX activity in potato tuber contrasts with that of green tomato fruit in which the pericarp contains a membrane-associated LOX activity that is kinetically distinct from a soluble counterpart and accounts for approximately 38% of the total soluble plus microsomal activity (38).

Both the microsomal and soluble activities have pH optima of 5.5, but only the microsomal fraction displayed some responsivenes to exogenous Ca\(^{2+}\). Whether this is due to a direct, specific effect of Ca\(^{2+}\) on the microsomal activity or to an indirect activation of secondary effectors in microsomes but not in the soluble fraction is unknown. Mulliez et al. (23) reported that highly purified preparations of the principal potato tuber 5-LOX isozyme are not activated by Ca\(^{2+}\). The results of a study by Ricker (31) in this laboratory also confirms that Ca\(^{2+}\) has no effect on the formation of AA hydroperoxides in tuber discs. Because only a small fraction of the total LOX activity modestly responded to Ca\(^{2+}\), and in
the light of the observations of Mulliez et al. (23) with highly purified LOX preparations, it seems unlikely that the reported Ca$^{2+}$ ion enhancement of the phytoalexin elicitor activity of AA (42) is due to a direct effect on LOX or to an enhanced generation of the initial LOX products of AA. In addition, the experiments with the β-glucan preparation indicate that its stimulation of AA elicitor activity is not at the level of LOX activity (22, 27).

The effects of the inhibitors on AA-induced LOX activity parallel to some degree their effects on sesquiterpenoid phytoalexin accumulation and other AA-induced responses such as lignification (6, 15). These compounds inhibit LOX through apparently different modes of action—PG is a free radical scavenger and SHAM competes with reducing substrate (28)—and therefore, these inhibitors undoubtedly react with sites other than LOX. Their effects on elicitor responses may only illustrate some of the general reaction chemistry involved. The mode of action of ABA in the context of our experiments is unclear, although ABA’s positive and negative effects on the expression of specific sets of genes and proteins in plants are well established (16). ABA could, therefore, function as a selective inhibitor or may induce a set of specific factors that suppress AA-induced responses.

In spite of the relatively high level of LOX in extracts of the inhibitor-treated discs, the inhibitor treatments strongly suppress other elicitor-induced responses (6, 28). This raises questions about the pathway sequence through which AA treatment impacts LOX and the other responses. The inhibition by PG and SHAM of the AA-induced increment in LOX activity suggests that this increment requires oxidation of AA or of a cell component(s) affected by AA treatment. One interpretation of these results is that, because the inhibitor-treated tissue has a relatively high capacity for peroxidizing AA, the inhibition derives in large part from an effect down-stream of the initial peroxidation of AA by LOX (i.e. a further metabolite of peroxidized AA or a secondary effector whose generation is highly sensitive to inhibition by PG and SHAM).

The effect of cycloheximide is intriguing because it did not inhibit LOX activity but rather enhanced activity similar to that following AA treatment. Under these conditions, cycloheximide completely suppresses browning, phytoalexin accumulation, and other responses to AA. The basis for the cycloheximide effect on LOX activity is unclear but may be due to inhibition of the synthesis of other proteins that participate in down-regulation or turnover of LOX. Although AA treatment causes an initial, transient suppression of protein synthesis in surface cells in tuber discs, the major portion of this inhibition appears to be due to depression in the uptake of the $[^3]H$leucine used for incorporation studies (37).

Hence, it seems unlikely that AA and cycloheximide operate through the same mechanism to enhance LOX activity, although this possibility should not be ruled out. The role of accessory proteins in regulation of 5-LOX is not without precedent. For example, the mammalian 5-LOX is activated by a hydrophobic 18-kD protein (10).

The damaging effects of reactive oxygen species to plant cells and their contribution to the HR have received increasing attention in studies of early events in plant-pathogen interaction (7). Lipid peroxides or their radical intermediates generated during the LOX reaction are also extremely disrup-

tive to the cell, capable of cooxidizing membrane lipids, carotenoids, and other components (34). In addition, singlet oxygen may be formed during the LOX reaction (34). In several plant-pathogen interactions, LOX activity increases during the HR. Yamamoto and Tani (41) correlated the appearance of two LOX isozymes in oat leaves following inoculation with an incompatible race of *Puccinia coronata avenae*, which did not increase in the compatible interaction.

LOX activation occurs in tomato leaves (21) and in French bean leaves (9) following inoculation with incompatible races of pathovars of *Pseudomonas syringae*, and its activation in tomato is preceded by an apparent induction of LOX mRNA (21). Rickauer et al. (30) reported the induction of LOX activity in tobacco cell cultures following treatment with an elicitor preparation from *Phytophthora parasitica var nicotiana*, although this induction, unlike our results, was strongly inhibited by cycloheximide. The activation of LOX with the formation of fungitoxic peroxo- and hydroxy-linoleic acids has been implicated in the resistance of rice to *Pyricularia oryzae* (24).

What is not clear from those studies, however, is whether LOX activation plays a causal role in the manifestation of symptoms and restriction of the pathogen or is a secondary and inconsequential response to hypersensitive cell collapse. Evidence presented by Peever and Higgins (25) suggests that LOX activation may not be simply a secondary response to tissue necrosis. Elicitors from *Cladosporium fulvum* induced LOX activity and lipid peroxidation in tomato leaflets incubated either in the dark or the light. However, necrosis only developed in elicitor-treated leaflets in the light, suggesting an uncoupling of the elicitor-induced LOX response and necrosis.

Our results bring into question how the potato LOX relates to specificity in the interaction with *P. infestans*. Although an incompatible race strongly and rapidly induces lipid peroxi-

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**Figure 9.** A scenario of the possible sequence of events for release and activation of eicosapolyenoic acids during infection of potato by *P. infestans* and subsequent induction of the host response cascade during hypersensitivity expression. Host responses include but are not limited to those mediated by transcriptional activation of genes.
The elicitor activity and rapid formation within 10 min of AA application (31) of 5-HPETE, the rapid enhancement of 5-LOX activity following AA treatment, and the evidence from earlier studies are consistent with the hypothesis that 5-LOX plays a role in the early signaling that leads to expression of defense-related processes. However, our study also indicates that the relationship between 5-LOX and arachidonic acid elicitation of the HR is still rather ambiguous, particularly in the light of the effects of the glucan and cycloheximide. Resolution of cause-and-effect relationships between this enzyme and expression of plant defense responses will be obtained with identification of an active LOX product of AA involved in elicitation, with specific probes (DNA and antibody) that discriminate among LOX isoforms, and through experiments that specifically alter the relevant LOX activities in vivo.

One possible scenario of the sequence of events from release of AA from fungal tissue to activation of the host response cascade is depicted in Figure 9. Although we were unable to demonstrate convincingly that 5-HPETE has elicitor activity in this system, the indirect evidence supports its participation in the response chain. Elements of the model are consistent with biochemical and microscopic studies of this interaction, and the model is intended to provide a conceptual framework for future research on the early processing and activation of eicosapolyenoic acids during fungal infection of plants.

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LITERATURE CITED


