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Indole-3-Acetic Acid Control on Acidic Oat Cell Wall Peroxidases

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Abstract. Incubation of oat coleoptile segments with 40 μM indoleacetic acid (IAA) induced a decrease of 35–60% in peroxidase activity at the cell wall compartment. Treatment with IAA also produced a similar decrease in the oxidation of NADH and IAA at the cell wall. Isoelectric focusing of ionic, covalent, and intercellular wall peroxidase fractions showed that acidic isoforms (pI 4.0–5.5) were reduced preferentially by IAA treatment. Marked differences were found between acidic and basic wall isoperoxidases in relation to their efficacy in the oxidation of IAA. A peroxidase fraction containing acidic isoforms oxidized IAA with a $V_{max}/s_{0.5}$ value of 2.4×10^{-2} $min^{-1} \cdot g\ fw^{-1}$, 4.0 times higher than that obtained for basic peroxidase isoforms (0.6×10^{-2} $min^{-1} \cdot g\ fw^{-1}$). In contrast, basic isoforms were more efficient than acidic isoperoxidases in the oxidation of coniferyl alcohol or ferulic acid with H_2O_2 (5.6 and 2.1 times, respectively). The levels of diferulate and lignin in the walls of oat coleoptile segments were not altered by treatment with IAA. The decrease in cell wall peroxidase activity by IAA was related more to reduced oxidative degradation of the hormone than to covalent cell wall cross-linking.

Key Words. IAA—Auxin—Wall peroxidases—Oat—*Avena sativa*—Diferulic acid—Lignin

The growth-stimulating effect of indoleacetic acid (IAA) on coleoptiles and other plant organs is well known (Cleland 1995), although the biochemical factors that modify cell wall extensibility in response to this phytohormone are poorly understood. Peroxidases are likely targets for IAA because they participate in the modulation of cell wall properties during plant growth. In fact, peroxidases catalyze the covalent cross-linking of wall polymers (Fry 1986, Iiyama et al. 1994), promoting the tightening of the wall as well as oxidizing IAA and thereby regulating the concentration of this hormone (Pedreño et al. 1988, Ros Barceló et al. 1990). Cross-linking of the cell wall occurs through the synthesis of diferulate bridges from ferulic acid residues bound to wall carbohydrates as well as by the oxidative coupling of cinnamoyl alcohol moieties to generate lignin (Fry 1986, Iiyama et al. 1994). Both of these reactions require hydrogen peroxide, which is generated by peroxidases through the oxidation of NADH or IAA by molecular oxygen (Ferrer et al. 1990, Mäder et al., 1980, Pedreño et al. 1988, Rojas et al. 1997).

Catabolism of IAA through oxidation has been demonstrated to be catalyzed by peroxidases in active growing tissues (Gordon and Henderson 1973, Ockerse and Mumford 1973, Ros Barceló et al. 1990). Several studies indicated that IAA decreased peroxidase activity in plant tissues in which this auxin induces cell elongation (Morrow and Jones 1986, Ockerse and Mumford 1973, Ros Barceló et al. 1989). The inhibited peroxidases include the IAA oxidase activity shown by certain peroxidase isoforms (Gordon and Henderson 1973, Ockerse and Mumford 1973). However, in these reports it is not possible to correlate reduced peroxidase activity by IAA with cell enlargement because most of the studies deal with total peroxidase activity present in crude extracts (Gordon and Henderson 1973, Morrow and Jones 1986, Ockerse and Mumford 1973); rarely has it been demonstrated that the measured peroxidase activity came from the cell walls without intracellular contamination (Morrow and Jones 1986, Ros Barceló et al. 1989). Peroxidases associated with the cell wall compartment are expected to participate in growth modulation because cell walls must be altered for cell enlargement to occur. In the cell wall peroxidases can be found in a variety of isoforms that differ in their electrophoretic mobilities (McDougall 1992, Ros Barceló et al. 1987, Van Huystee

Abbreviations: IAA, indoleacetic acid; *o*-PDA, *o*-phenylenediamine; DFA, diferulic acid; HPLC, high performance liquid chromatography; fw, fresh weight.

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1987). These isoforms can be associated to the walls by ionic interactions or be more tightly (covalently) bound, being released only by enzymatic degradation of wall polymers. Soluble isoperoxidases can also be found in the intercellular cell wall compartment. All of these peroxidase fractions could be potentially altered by IAA. In this paper we describe the effect of this hormone on three different wall peroxidase fractions obtained from oat coleoptiles, as a contribution to the understanding of peroxidase participation in IAA-induced plant cell wall extension. We found that IAA induced a selective decrease of acidic wall-bound peroxidases which appears to be related to reduced IAA oxidation rather than to altered wall cross-linking.

Materials and Methods

Plants and Incubations with IAA

Six-day-old coleoptiles of *Avena sativa* L. cv. Nehuen were grown at 22–25°C in the dark. Segments of 1–1.5 cm were cut 3 mm under the tip and washed extensively with distilled water. The segments were incubated at 24°C with 40 μM IAA in malic buffer (5 mM, pH 5.5) and 1% p/v sucrose in aerated plastic tubes, changing the solution every 6 h. After different times of incubation in the dark, growth was evaluated by the increment in the fresh weight of the coleoptile segments.

Separation of Cell Wall Peroxidase Fractions

Oat coleoptile segments were ground in a mortar with 50 mM Tris-HCl, pH 7.2, containing 0.1 mM phenylmethylsulfonyl fluoride, 5% insoluble polyvinylpolypyrrolidone, 5 mM $MgCl_2$, and sand. Cell walls were collected by centrifugation at 1,000 ×*g* for 5 min and washed with Triton X-100 according to Ros Barceló et al. (1987). The purity of the cell wall fragments obtained was evaluated by the enzymatic activities of biochemical markers for mithochondria (cytochrome *c* oxidase, EC 1.9.3.1), endoplasmic reticulum (NADH-cytochrome *c* reductase, EC 1.6.99.3), and plasma membrane (AMP-nucleotidase, EC 3.1.3.5), as described by Briskin et al. (1987). Ionically bound (ionic) cell wall peroxidases were obtained by incubation of the wall pellet with 1 M KCl while covalently bound (covalent) cell wall peroxidases were solubilized from the remaining pellet by treatment with pectinase plus cellulase (Ros Barceló et al. 1987) including 2 mM benzamidine and 1 μM pepstatin A in the reaction mixture. The intercellular peroxidase content was measured by vacuum infiltration of the coleoptile segments with 10 mM Tris-HCl, pH 7.4, containing 20 mM $CaCl_2$ followed by centrifugation for 5 min at 800 ×*g*. There was no contamination with intracellular material, as demonstrated by the absence of glucose-6-phosphate dehydrogenase activity (Ros Barceló et al. 1987). Acidic and basic peroxidases were isolated from the ionic wall fraction by column chromatography in carboxymethyl Sepharose at pH 6.4 in 15 mM phosphate buffer. The fraction that was not bound to the column under these conditions contained basic peroxidases mainly of pI 9.6 (basic fraction, 43% of total peroxidase activity applied to the column), whereas bound activity was eluted with 1 M KCl and contained acidic peroxidases mainly of pI 4.6 (acidic fraction, 29% of total peroxidase activity).

Peroxidase Assays

All assays were carried out in 1-mL cuvettes in a Shimadzu UV-120 spectrophotometer at 24°C. The assays with *o*-phenylendiamine (*o*-PDA, 2 mM), ferulic acid, or coniferyl alcohol (80 μM) were carried out in citrate buffer (0.1 M, pH 4.5) with 0.5 mM H_2O_2. The absorbance change with these substrates was measured at 450 nm (ϵ = 1.05 $mM^{-1}cm^{-1}$), 310 nm (ϵ = 16 $mM^{-1}cm^{-1}$), and 260 nm (ϵ = 2.2 $mM^{-1}cm^{-1}$), respectively. NADH oxidase activity was monitored by the decrease in absorbance at 340 nm (ϵ = 6.02 $mM^{-1}cm^{-1}$) in an assay mixture containing 50 μM $MnSO_4$, 70 μM *p*-coumaric acid, and 0.16 mM NADH in phosphate buffer (0.1 M, pH 7.0). Rates were calculated from the slope of the progress curves, whereas half-saturating substrate concentrations ($s_{0.5}$) and V_{max} values were obtained by fitting the rate data for different substrate concentrations to hyperbolic functions by nonlinear regression analysis. IAA oxidation by oat cell wall fractions was recorded at 250 nm in a reaction medium containing 50 μM $MnSO_4$, 80 μM *p*-coumaric acid, and variable IAA concentrations in phosphate buffer (0.1 M, pH 6.2). An extinction coefficient of 18.75 $mM^{-1}cm^{-1}$ was utilized for the oxidation product of IAA (García Florenciano et al. 1991).

Determination of DFA and Lignin Levels in Oat Cell Walls

All procedures were carried out under fluorescent light. Oat coleoptiles were ground in a mortar with liquid nitrogen, and the resulting fine powder was suspended in 1.5 mL of 2% (w/v) Triton X-100, 50 mM azide, and 1 M KCl. The mixture was stirred vigorously for 15 min. After centrifugation (14,000 rpm, 5 min) the pellet was washed consecutively with MeOH (twice), H_2O, 1% (w/v) SDS, H_2O (twice), EtOH, MeOH/$CHCl_3$ (1:1 v/v), EtOH and H_2O (twice). The remaining insoluble material was incubated with 5 mL of α-amylase (1 mg/mL) in 50 mM Tris, pH 7.0, for 6 h and then with 5 mL of Pronase E (0.5 mg/mL) in the same buffer overnight. Finally the cell walls were washed again with H_2O, MeOH, MeOH/$CHCl_3$ (1:1 v/v) and dried at 40°C. For diferulate quantification, the phenolics ester-linked to wall polysaccharides were released by alkaline hydrolysis. Dried cell walls (20–40 mg) were treated at room temperature for 20 h with 2 M NaOH (5 mL degassed) in vials sealed under N_2. A solution of caffeic (*E*-3,4-OH-cinnamic) acid was added to the vials as internal standard. After hydrolysis, the mixture was centrifuged, and the alkaline fraction was acidified to pH 2.0 with HCl and then extracted with Et_2O (4 × 10 mL). The combined ethereal extracts were vacuum dried and stored in the dark. Samples were analyzed by HPLC on a reversed phase Waters Nova-Pak C_{18} (150 × 3.9 mm inner diameter) column in a Waters model 600E system controller fitted with a Waters Ultra Wisp model 715 sample processor and a photodiode array detector (Waters model 990^+). Monitoring was at 310 nm. The flow rate was 0.9 mL/min, and the elution was performed with a MeCN convex gradient against 3% HOAc. After sample injection, the MeCN was kept in 5% for 5 min, then increased to 10% in 15 min, and to 20% in an additional 25 min. The proportion of MeCN was held at 20% for 10 min, then increased to 50% in 10 min, and after 2 min, the MeCN proportion was brought back to 5% for an additional 13 min. The quantities of DFA were calculated according to the yield of the internal standard and by calibration with authentic DFA. Lignin was determined in the saponified cell walls by the method of Iiyama and Wallis (1990).

Isoelectric Focusing

Pharmalyte ampholytes (Amersham Pharmacia Biotech), in a pH range of 3–10, were utilized in polyacrylamide gels using a Bio-Rad 111 mini-IEF chamber according to the manufacturer's specifications. Before the run, wall fraction samples were desalted and concentrated on Centricon tubes (cutoff 10,000, from Amicon) and 16.7–25 nkat of

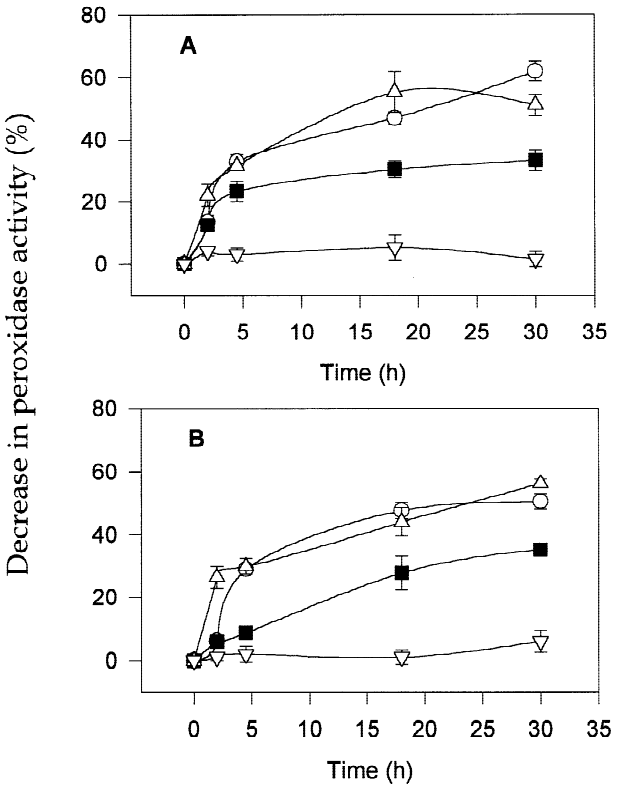

Fig. 1. Decrease in wall-bound peroxidase levels upon treatment of oat coleoptiles with 40 μM IAA. Peroxidase activity was assayed with coniferyl alcohol and H_2O_2 (*A*) or NADH and O_2 in the presence of *p*-coumaric acid (*B*) as described in the Materials and Methods section. ○, ionic fraction; ■, covalent fraction; △, intercellular fraction, ▽, intercellular fraction from coleoptiles treated with benzoic acid. The decrease in peroxidase activity is expressed as a percent of the respective controls (incubated in the absence of IAA). Activity values were normalized per g of tissue dry weight. Data are the means ± S.E. of five independent samples.

peroxidase measured with *o*-PDA and H_2O_2 was applied to the gels. After the run, the gels were stained with 5 mM 4-methoxy-1-naphthol and 0.04% (v/v) H_2O_2 in citrate buffer (0.1 M, pH 4.5).

Results and Discussion

Decrease in Wall Peroxidase Activity by IAA

Peroxidase activity released from the cell wall compartment of IAA-treated oat coleoptiles showed a marked decrease relative to control tissue incubated in the absence of this auxin (Fig. 1). This peroxidase activity came exclusively from the cell walls without intracellular contamination because our purified cell wall fractions were devoid of enzymatic activity marker of mitochondria, endoplasmic reticulum, and plasma membrane. After incubating the coleoptiles for 18 h with 40 μM IAA, wall peroxidase activity was 30–50% lower than in control incubations. This decrease was found for the ionically and covalently bound peroxidase fractions as well as for peroxidases in the infiltration fluid (intercellular fraction) and occurred when peroxidase activity was measured with coniferyl alcohol (Fig. 1*A*) or with NADH (Fig. 1*B*) as substrate. IAA oxidase activity also showed a similar decrease in IAA-treated coleoptiles. In the intercellular fraction, the decrease in IAA oxidase was 18% after 1.5 h and 58% and 65% after 12 h and 18 h of IAA treatment, respectively. The observed inhibition leveled off at 18 h and remained at 65% up to 30 h from the start of the treatment. Benzoic acid, an aromatic carboxylic acid with a structure similar to that of IAA but without auxin activity, did not alter wall peroxidase activity when added into the incubation solution (Fig. 1), thus suggesting that the observed effect was specific for compounds with IAA-like biological activity.

The reduction in peroxidase activity by exogenously applied IAA has been reported in several plant systems (Gordon and Henderson 1973, Morrow and Jones 1986, Ockerse et al. 1965, Ockerse and Mumford 1973, Ros Barceló et al. 1989), but only a few reports describe the effect of this auxin on peroxidases from the cell walls (Morrow and Jones 1986, Ros Barceló et al. 1989). In pea internode sections, intercellular peroxidase activity was decreased by IAA treatment, an effect that correlated inversely with the elongation rate (Morrow and Jones 1986), and application of IAA or 2,4 dichlorophenoxiacetic acid to exponentially growing lupin hypocotyls promoted a reduction in the level of free intercellular peroxidases (Ros Barceló et al. 1989). We found that the decrease in oat wall peroxidases by IAA (Fig. 1) paralleled coleoptile elongation (Fig. 2) and correlated with the enhanced growth rate induced by this hormone. The growth-stimulatory effect was observed from 1 h after treatment and leveled off after 10 h, with a time course similar to that observed for peroxidase inhibition. Significant differences in the degree of inhibition of the activities of ionic, covalent, and intercellular peroxidases were observed after IAA treatment (Fig. 1). For the ionic and intercellular fractions, the decrease in peroxidase activity was about 50% after 20 h of treatment, whereas only 30% inhibition was found for the covalent wall peroxidases that are bound tightly to the cell walls. This difference could be attributed to a slower turnover of covalent peroxidases relative to weakly bound isoforms caused by trapping of the former in the cell wall matrix or binding of peroxidases from the intercellular fraction to the walls as a result of IAA activity. In fact, IAA and acidic buffers have been reported to decrease peroxidase activity in the intercellular fluid by promoting their binding into the cell walls (Ros Barceló et al. 1988, 1989). The auxin effect on oat cell wall peroxidases reported here is consistent with a role for these enzymes in the regulation of the mechanical properties of the cell wall (Fry 1986, Iiyama et al. 1994); the inhibition of wall-

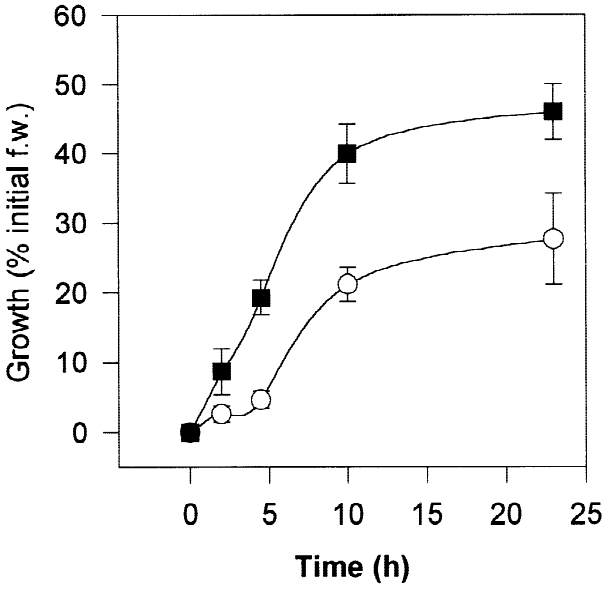

Fig. 2. Time course of growth stimulation by IAA in oat coleoptiles. Coleoptile segments were incubated as described in Materials and Methods with (■) or without (○) 40 μM IAA. Growth was expressed as % of the initial fresh weight. Data are means ± S.E. of five independent samples.

bound peroxidases including the IAA oxidase activity could induce either a decrease in wall cross-linking or a reduction in auxin catabolism, both reactions that could contribute to the promotion of wall extension and, therefore, tissue growth.

Changes in Peroxidase Isoform Patterns Induced by IAA

The isoperoxidase patterns in the ionic, covalent, and intercellular wall fractions from the oat coleoptiles were altered by treatment with IAA (Fig. 3). The ionic and covalent fractions contained acidic and basic peroxidases as the main isoforms (pI values of 4.6 and 9.6 for the ionic fraction and 4.3 and 8.0 for the covalent fraction), whereas acid and neutral isoperoxidases (pI 4.6 and 6.2) were the main isoforms in the intercellular fraction. Incubation of the coleoptiles with 40 μM IAA for 20 h decreased the activity of the acidic peroxidase isoforms (pI 4–5) present in the cell wall fractions (Fig. 3). This response was observed for the ionic, covalent, and intercellular peroxidases and indicated preferential control of acidic isoforms by IAA. Furthermore, the basic isoform with pI 9.6 appeared in the intercellular fraction upon IAA treatment, whereas the proportion of this basic isoform in the ionic fraction increased in relation to total peroxidase activity in auxin-treated samples (Fig. 3). The peroxidase pattern in the intercellular fraction after isoelectric focusing was not altered by treatment with ben-

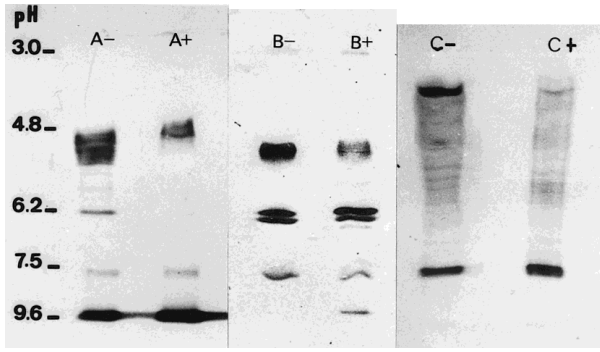

Fig. 3. Peroxidase isoforms in cell wall fractions from oat coleoptiles incubated with (+) or without (−) 40 μM IAA for 20 h. *A,* ionic fraction; *B,* intercellular fraction; *C,* covalent fraction.

zoic acid (not shown), demonstrating the specific effect of IAA on peroxidase isoforms. Induction and repression of isoperoxidases by IAA have been already reported (Gordon and Henderson 1973, Morrow and Jones 1986, Ockerse and Mumford 1973, Ockerse et al. 1965, Ros Barceló et al. 1989). In lupin hypocotyls, intercellular acidic isoperoxidases are under auxin control (Ros Barceló et al. 1989) with a short term reduction in their levels upon application of IAA. On the other hand, in crude extracts from oat coleoptiles there is evidence of a decrease in two anodic peroxidase isoforms and induction of two cathodic isoperoxidases by IAA. However, these changes in peroxidases could not be related to cell wall expansion and growth because they were mainly intracellular (Gordon and Henderson 1973). Also, IAA has been shown to induce both qualitative and quantitative changes in the isoperoxidase patterns of pea stem extracts (Ockerse and Mumford 1973, Ockerse et al. 1965). A specific isoperoxidase corresponding to a basic isoform that appears as the tissue elongates and ages was repressed by IAA (Ockerse and Mumford 1973, Ockerse et al. 1965). Recently, direct evidence has been found for auxin control of the synthesis of acidic peroxidase isoforms (Klotz and Lagrimini 1996). The promoter activity of an acidic isoperoxidase was strongly suppressed by IAA in tobacco mesophyl protoplasts (Klotz and Lagrimini 1996). This acidic isoform has been implicated in lignin formation because it is the major peroxidase in the wall of stem tissue (Lagrimini et al. 1987), and it may also have a role in IAA metabolism (Lagrimini et al. 1997). The inhibitory effect on peroxidase gene expression was found to be specific for auxins and could not be obtained with other plant hormones such as benzyladenine, gibberellins, ethylene, or abscisic acid (Klotz and Lagrimini 1996). Thus, reduced synthesis of mainly acidic oat cell wall peroxidases, induced by IAA, could contribute to the decrease in peroxidase activity we find, even if decreased transport into the walls (Fry 1980) or enzyme inactivation through modification cannot be ruled out.

Table 1. Apparent kinetic parameters of acidic and basic isoforms from ionically wall-bound peroxidase of oat coleoptiles.

Substrate and isoforms	V_{max} (μM/min)[a]	$s_{0.5}$ (μ*M*)	$V_{max}/s_{0.5}$ ($min^{-1} \times 10^{-2}$)
Ferulic acid			
Acid	11.4 ± 0.9	59.2 ± 3.1	19.3 ± 2.5
Basic	37.5 ± 1.9	91.1 ± 6.3	41.2 ± 4.9
Coniferyl alcohol			
Acid	57.4 ± 2.9	454.4 ± 36.9	12.6 ± 1.7
Basic	234.6 ± 14.5	330.4 ± 26.7	71.0 ± 9.0
H_2O_2[b]			
Acid	1.7 ± 0.1	20.2 ± 1.6	8.4 ± 1.2
Basic	3.5 ± 0.1	12.8 ± 1.1	27.3 ± 3.1
IAA			
Acid	1.4 ± 0.1	57.5 ± 5.5	2.4 ± 0.4
Basic	2.2 ± 0.2	381.3 ± 45.2	0.6 ± 0.1

[a]Rates are normalized per g fresh weight.
[b]Assayed with 80 μM ferulic acid as reductant.

IAA Oxidation by Acidic and Basic Wall Isoperoxidases

Acidic and basic isoperoxidases from the ionic fraction of untreated coleoptiles were isolated by cationic exchange chromatography, and their substrate specificity was investigated (Table 1). Both groups of isoforms showed marked differences in the utilization of IAA and of the phenolic substrates coniferyl alcohol and ferulic acid (Table 1). The fraction containing mainly acidic isoperoxidases (pI 4.6) oxidized IAA in the presence of O_2 and *p*-coumaric acid with a $V_{max}/s_{0.5}$ value of 2.4×10^{-2} $min^{-1} \cdot g\ fw^{-1}$, whereas the fraction that contained mainly basic isoforms (pI 9.6) catalyzed the same reaction with a $V_{max}/s_{0.5}$ value of 0.6×10^{-2} $min^{-1} \cdot g\ fw^{-1}$, 4.0 times lower than that obtained for the acidic fraction. In contrast, coniferyl alcohol, ferulic acid, and H_2O_2 were utilized at higher rates by basic isoperoxidases (Table 1) with $V_{max}/s_{0.5}$ values that are 5.6, 2.1, and 3.3 times higher, respectively, than those obtained for the acidic fraction. Similar results were also obtained when V_{max} values were normalized by the heme content (A_{403}) or by the units of peroxidase activity in the sample measured with *o*-PDA and H_2O_2. These results are in agreement with our finding that ionically bound wall peroxidases show higher rates of utilization of the phenolic substrates than covalently bound peroxidases that lack the basic (pI 9.6) isoform. It has been suggested that wall isoperoxidases would modulate cell expansion by regulating auxin levels (Gordon and Henderson 1973). The differences we find in the utilization of IAA by acidic and basic wall peroxidases indicate that the former are the more important for IAA oxidation in the oat system, so explaining the preferential inhibition of this group of isoforms by auxin (Fig. 3). Catalysis of IAA oxidation by oat wall peroxidases and a decrease of peroxidase activity by IAA suggest a role for this mutual interaction in the regulation of growth. In fact, inhibition by IAA of peroxidases involved in IAA inactivation would contribute to the enhanced elongation response induced by this exogenously added auxin (Fig. 2). It is likely that peroxidase inhibition by IAA depends on auxin concentration and would be greater in tissues containing high levels of IAA which are actively growing. In agreement with this is has been shown that IAA oxidation rates as well as peroxidase activities vary inversely with IAA levels in actively growing regions of lupin hypocotyls near the cotyledons (Ferrer et al. 1991, Sánchez-Bravo et al. 1988). Furthermore, IAA oxidation mediated by peroxidases generates highly reactive free radicals and active oxygen species derived from H_2O_2 (Candeias et al. 1995, 1996, Ferrer et al. 1990, Metodiewa et al. 1992, Pedreño et al. 1988) which are potentially harmful for the coleoptile. Thus, the decrease of peroxidase activities, including IAA oxidase activity, at the cell wall by IAA could serve as a control mechanism to avoid oxidative damage in this actively growing tissue in the presence of high IAA concentrations. In fact, evidence for oxidative damage in membrane lipids by peroxidase-catalyzed IAA oxidation has already been described (Candeias et al. 1995, 1996).

Diferulic Acid and Lignin Levels in the Walls of IAA-Treated Coleoptiles

The correlation between growth and decrease in cell wall peroxidase activity after IAA treatment (Figs. 1 and 2) was not reflected in the lignin or DFA levels in the coleoptile walls. Incubation of oat coleoptile segments for up to 20 h with 40 μM IAA did not alter diferulate accumulation in the cell walls relative to the respective controls (Fig. 4*A*). The same was found for lignin wall content (Fig. 4*B*). The levels of diferulate or lignin increased up to 23 h after treatment with IAA, but this increase was the same in the coleoptiles incubated in the absence of exogenous hormone. This indicates that peroxidase activity is not a limiting factor in determining the accumulation of these products in the walls. The linear increase in wall DFA content, mediated by peroxidases, is likely to be associated with the covalent cross-linking necessary to support the cell wall structure upon incorporation of new material into the existing walls during elongation. Also, the DFA content could be related to the cessation of growth as suggested by the inverse correlation already reported between DFA levels and wall extensibility (Tan et al. 1991, 1992). Lignin content has been inversely related with growth (Whitmore 1971) and thus would also contribute to growth limitation under the conditions used in our experiments. However, the absence of a difference in the levels of DFA and lignin between control and IAA-treated coleoptiles supports an

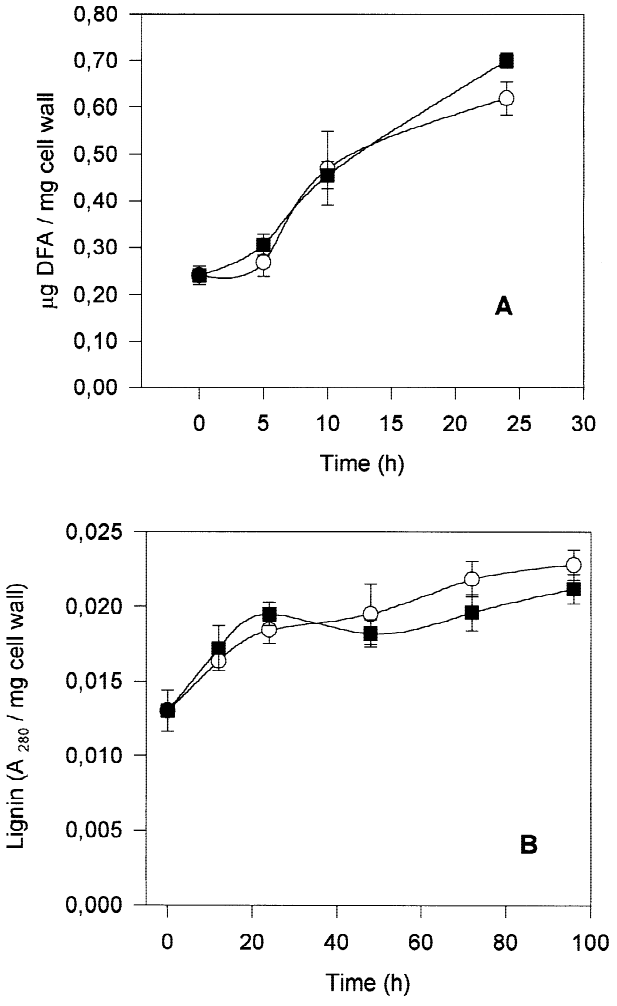

Fig. 4. Diferulate (*A*) and lignin (*B*) levels in cell walls from oat coleoptiles incubated in the absence (○) or in presence (■) of 40 µM IAA. Data are the means ± SE of five independent samples.

independent control of wall loosening and wall stiffening processes during cell wall extension, as proposed previously in maize coleoptiles (Hohl et al. 1995, Müsel et al. 1997). In agreement with our results, it is likely that IAA can affect wall loosening without altering wall stiffening processes as already described elsewhere (Müsel et al. 1997).

The results reported here suggest that the effect of exogenous IAA on peroxidase activity in oat coleoptiles, at a concentration that gives maximum elongation, could be related to IAA oxidation rather than to cell wall cross-linking. A likely early response of this actively growing tissue toward exogenous IAA would be to diminish IAA oxidation through inhibition of peroxidase isoforms involved in its catabolism. This response would contribute to the level of the hormone as well as to the reduction of oxidative damage derived from the H_2O_2 generated from IAA oxidation. The preferential decrease of acid peroxidase isoforms associated with the coleoptile cell walls, which oxidize IAA at higher rates than basic isoperoxidases, supports this idea. Future experiments on over- and underexpression of acidic isoforms being regulated by IAA would contribute to the understanding of their role in avoiding oxidative damage derived from IAA catabolism.

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References

Briskin D, Leonard R, Hodges T (1987) Isolation of the plasma membrane: Membrane markers and general principles. Methods Enzymol 148:542–568

Candeias LP, Folkes LK, Porssa M, Parrick J, Wardman P (1995) Enhancement of lipid peroxidation by indole-3-acetic acid and derivatives: Substituent effects. Free Radical Res 23:403–418

Candeias LP, Folkes LK, Wardman P (1996) Enhancement of peroxidase-induced lipid peroxidation by indole-3-acetic acid: Effect of antioxidants. Redox Rep 2:141–147

Cleland RE (1995) Auxin and cell elongation. In: Davies PJ (ed) Plant hormones. Kluwer Academic Publishers, Netherlands, pp 214–227

Ferrer MA, Pedreño MA, Muñoz R, Ros Barceló A (1990) Oxidation of coniferyl alcohol by cell wall peroxidases at the expense of indole-3-acetic acid and O_2. FEBS Lett 276:127–130

Ferrer MA, Pedreño MA, Muñoz R, Ros Barceló A (1991) Soluble peroxidase gradients in lupin hypocotyls and the control of the level of polarly transported indole-3yl-acetic acid. J Plant Growth Regul 10:139–146

Fry SC (1980) Gibberellin-controlled pectinic acid and protein secretion in growing cells. Phytochemistry 19:735–740

Fry SC (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annu Rev Plant Physiol 37:165–186

García Florenciano E, Calderón A, Pedreño MA, Muñoz R, Ros Barceló A (1991) The vacuolar localization of basic isoperoxidases in grapevine suspension cell cultures and its significance in indole-3-acetic acid catabolism. Plant Growth Regul 10:125–138

Gordon WR, Henderson JHM (1973) Isoperoxidases of (IAA oxidase) oxidase in oat coleoptiles. Can J Bot 51:2047–2052

Hohl M, Greiner H, Schopfer P (1995) The cryptic growth response of maize coleoptiles and its relationship to H_2O_2-dependent cell wall stiffening. Physiol Plant 94:491–498

Iiyama K, Lam TB-T, Stone BA (1994) Covalent cross-links in the cell wall. Plant Physiol 104:315–320

Iiyama K, Wallis AF (1990) Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. J Sci Food Agric 51:145–161

Klotz KL, Lagrimini LM (1996) Phytohormone control of the tobacco anionic peroxidase promoter. Plant Mol Biol 31:565–573

Lagrimini LM, Burkhart W, Moyer M, Rothstein S (1987) Molecular cloning of complementary DNA encoding the lignin-forming

peroxidase from tobacco: Molecular analysis and tissue-specific expression. Proc Natl Acad Sci USA 84:7542–7546

Lagrimini LM, Joly RJ, Dunlap JR, Liu TY (1997) The consequence of peroxidase overexpression in transgenic plants on root growth and development. Plant Mol Biol 33:887–895

Mäder M, Ungemach J, Schloss P (1980) The role of peroxidase isoenzyme groups of *Nicotiana tabacum* in hydrogen peroxide formation. Planta 147:467–470

McDougall GJ (1992) Changes in cell wall-associated peroxidases during the lignification of flax fibres. Phytochemistry 31:3385–3389

Metodiewa D, de Melo MP, Escobar JA, Cilento G, Dunford HB (1992) Horseradish peroxidase-catalyzed aerobic oxidation and peroxidation of indole-3-acetic acid. Arch Biochem Biophys 296:27–33

Morrow DL, Jones RL (1986) Localization and partial characterization of the extracellular proteins centrifuged from pea internodes. Physiol Plant 67:397–407

Müsel G, Schindler T, Bergfeld R, Ruel K, Jacquet G, Lapierre C, Speth V, Schopfer P (1997) Structure and distribution of lignin in primary and secondary cell walls of maize coleoptiles analyzed by chemical and immunological probes. Planta 201:146–159

Ockerse R, Mumford LM (1973) The regulation of peroxidase activity by gibberellin and auxin in pea stem segments. Can J Bot 51: 2237–2242

Ockerse R, Siegel BZ, Galston AW (1965) Hormone-induced repression of a peroxidase isozyme in plant tissue. Science 151:452–453

Pedreño MA, Muñoz R, Sabater F (1988) Oxidase and peroxidase activities of a cell wall-bound peroxidase acting on indoleacetic acid: Control by *p*-coumaric acid. Biochem Int 16:465–475

Rojas MC, Perez F, González LF (1997) Stimulatory effect of DIMBOA on NADH oxidation catalyzed by horseradish peroxidase. Phytochemistry 46:11–15

Ros Barceló A, Muñoz R, Sabater F (1987) Lupin peroxidases. I. Isolation and characterization of cell wall-bound isoperoxidase activity. Physiol Plant 71:448–454

Ros Barceló A, Pedreño MA, Ferrer MA, Sabater F, Muñoz R (1990) Indole-3-methanol is the main product of the oxidation of indole-3-acetic acid catalyzed by two cytosolic basic isoperoxidases from *Lupinus.* Planta 181:448–450

Ros Barceló A, Pedreño MA, Muñoz R, Sabater F (1988) Lupin peroxidases. II. Binding of acidic isoperoxidases to cell walls. Physiol Plant 73:238–244

Ros Barceló A, Pedreño MA, Muñoz R, Sabater F (1989) Physiological significance of the binding of acidic isoperoxidases to cell walls of lupin. Physiol Plant 75:267–274

Sánchez-Bravo J, Acosta M, Sabater F (1988) In vivo metabolism of labelled indole-3-acetic acid during polar transport in etiolated hypocotyls of *Lupinus albus:* Relationship with growth. Plant Growth Regul 7:271–288

Tan K-S, Hoson T, Masuda Y, Kamisaka S (1991) Correlation between cell wall extensibility and the content of diferulic and ferulic acids in cell walls of *Oryza sativa* coleoptiles grown under water and in air. Physiol Plant 83:397–403

Tan K-S, Hoson T, Masuda Y, Kamisaka S (1992) Involvement of cell wall-bound diferulic acid in light-induced decrease in growth rate and cell wall extensibility of Oryza coleoptiles. Plant Cell Physiol 33:103–108

Van Huystee RB (1987) Some molecular aspects of plant peroxidase biosynthetic studies. Annu Rev Plant Physiol 38:205–219

Whitmore FW (1971) Lignin formation in wheat coleoptile cell walls: A possible limitation of cell growth. Plant Physiol 48:596–602