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Role of Apoplastic and Cell-Wall Peroxidases on the Stimulation of Root Elongation by Ascorbate[1]

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Elongation of onion (*Allium cepa* L.) roots was highly stimulated by ascorbate (ASC) and its natural precursor l-galactone-γ-lactone (GL). When incubation media were supplemented with lycorine (Lyc), an inhibitor of the ASC biosynthesis, root growth was negligible even in the presence of ASC or GL. ASC completely inhibited in vitro guaiacol peroxidase activities that were isolated from both the apoplast and the cell wall. However, ferulic-acid-dependent peroxidase from the cell wall was partially inhibited by ASC, whereas ferulic acid peroxidase activity from the apoplastic fluid was completely inhibited by ASC as long as ASC was present in the assay medium. ASC content in cells was increased by preincubations with ASC or GL, whereas Lyc reduced it. On the other hand, ASC or GL treatments decreased both apoplast and cell-wall-bound peroxidase activities, whereas Lyc had a slight stimulating effect. These results are discussed on the basis of a possible control of root elongation by ASC via its action on peroxidases that are involved in the regulation of cell-wall extensibility.

ASC and its free radical AFR stimulate the elongation of onion (*Allium cepa* L.) roots in parallel to a high vacuolization of meristematic cells (Hidalgo et al., 1989; Navas, 1991; González-Reyes et al., 1994b). ASC is required for the progression of the G1 and G2 phases of the cell cycle (Liso et al., 1984). Current explanations for these observations include (a) the hyperpolarization of the plasma membrane and acidification of the apoplast induced by ASC and AFR (González-Reyes et al., 1992) after the activation of a plasmalemma NADH-AFR oxidoreductase activity that stimulates nutrient intake (Gonzalez-Reyes et al., 1994b), and (b) the maintenance, in a reduced state of Hyp-rich proteins, that is needed for the progression of the cell cycle (Arrigoni et al., 1977; De Gara et al., 1991a). Two recent reviews analyze these hypotheses in detail (Arrigoni, 1994; Córdoba and González-Reyes, 1994).

Root growth and elongation leads to an irreversible increase in cell volume. Thus, the cell-wall architecture is modified in such a way that a relaxation of the crossed bonds that link several components of the cell wall must occur. Cell-wall loosening allows the increase of the cell surface and the intake of water by the protoplast, and changes in the cell-wall architecture during cell elongation have been recently reported (McCann and Roberts, 1994).

Cell-wall-bound and/or apoplastic peroxidases have been involved in the formation of isodityrosine bonds between glycoproteins as extensin, or diferulate bridges between polysaccharide polymers (Biggs and Fry, 1987). These peroxidase-driven cross-linking reactions of cell-wall polymers stiffened the cell wall during growth, thus reducing the rate of elongation (Fry, 1986). To comprehend the regulation of cell-wall extensibility during growth, focus must be placed on the biological control of peroxidase activities at the cell wall. This includes the synthesis and secretion of peroxidases, the supply of substrates as phenols or H_2O_2, and the levels of peroxidase-modulating agents (see also Biggs and Fry, 1987).

ASC is synthesized in plants from Glc and acts as a free-radical scavenger in a variety of cell compartments (Foyer et al., 1991), with its concentration being dependent on the developmental stage (Arrigoni et al., 1992). ASC is also a common antioxidant component in the apoplast (Castillo and Greppin, 1988; Polle et al., 1990; Takahama and Oniki, 1992; Luwe et al., 1993) and is known to be a strong in vitro inhibitor of peroxidases (Takahama and Oniki, 1992, 1994; Takahama, 1993a, 1993b). However, a question concerning the in vivo regulation of cell-wall extensibility via ASC-dependent peroxidase-driven reactions has yet to be answered.

In this paper we have investigated the stimulation of onion root elongation by ASC in relation to its concentration at the root and its regulatory role on apoplastic and cell-wall-bound peroxidases. The data presented here show a significant negative correlation between cellular ASC levels and apoplastic and cell-wall-bound peroxidase activities, suggesting that root elongation in onions is stimulated by the ASC-inhibited peroxidases that are involved in the cross-linking reactions at the cell wall.

MATERIALS AND METHODS

Growth Conditions

Onion (*Allium cepa* L.) bulbs were grown hydroponically in darkness so that only their basal plates were immersed

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Abbreviations: AFR, ascorbate free radical; ASC, ascorbate; DHA, dehydroascorbate; GL, L-galactone-γ-lactone; Lyc, lycorine.

in water. Temperature was maintained at 25°C, and water was permanently aerated (10–20 mL air/min) with an air pump (Rema, Madrid, Spain) and renewed every 24 h. After 72 h, when most of the roots had reached 3 cm in length, bulbs were washed and subjected to different treatments as indicated.

Several agents were used in experiments. Sodium salts of ASC and DHA were used at final concentrations of 1 mM. GL was diluted in water and used at a final concentration of 2 mM. Lyc, a generous gift from Prof. O. Arrigoni and Dr. L. De Gara (University of Bari, Italy), was prepared as a 1 mM stock solution and used at a final concentration of 50 μM. In all in vivo treatments culture media were buffered at pH 6.0 with 10 mM sodium phosphate. Some bulbs remained in phosphate buffer as controls. All experiments were performed in the dark.

Root Growth Measurement

At the beginning of each treatment several roots were removed from the bulb, and only 10 to 12 roots of similar length remained attached to the onion crown. Once treatments began, lengths were carefully measured every 5 h using a flexible plastic ruler. Elongation experiments were repeated three times using at least five bulbs per treatment.

Isolation of Apoplastic Fluid, Cell-Wall Fragments, and Soluble Cytosolic Components

After 15 h of preincubation with working solutions, roots were thoroughly washed in 10 mM sodium phosphate and processed to obtain apoplastic fluid and cell-wall fragments according to Takahama and Oniki (1992, 1994). Briefly, apoplastic soluble components were obtained from 15-mm apical root fragments and vacuum-infiltrated in 10 mM sodium phosphate, pH 6.0, at 4°C for 5 min, followed by centrifugation at 1500*g* for 5 min. The activity of the Glc-6-P dehydrogenase was determined according to Löhr and Waller (1974) as a control of cytosolic contamination. The enzyme activity in the apoplastic fluid was lower than 2% of the whole cell, indicating a negligible cytosolic contamination.

Once apoplastic fluids were removed, root segments were cut into 2-mm pieces with a razor blade and homogenized in 10 mM sodium phosphate, pH 6.0, with an Ultra-Turrax (Janke & Kunkel, Staufen, Germany) for 1 min at 4°C. Roots were ground in 5% methaphosphoric acid when ASC had to be estimated in soluble fractions (De Gara et al., 1991a). The homogenate was filtered through four cheesecloth layers and centrifuged at 5000*g* for 5 min. The supernatant represents the soluble cytosolic fraction. The pellet was resuspended in the same buffer and centrifuged again. Washing was repeated twice, and the resulting pellet was resuspended in 10 mM phosphate buffer, pH 6.0 (cell-wall preparation).

Assays of Peroxidase Activities

Guaiacol- and ferulic-acid-dependent peroxidases were determined in apoplastic fluid and cell-wall preparations according to Zheng and Van Huystee (1992). Reactions were developed in a final volume of 1 mL containing 20 to 50 μg of sample protein, 10 mM sodium phosphate (pH 6.0), 0.1 mL of 0.3% (v/v) H_2O_2, and 0.1 mL of 1% (v/v) guaiacol or 0.1 mM ferulic acid. Reactions were followed spectrophotometrically for 5 min at 30°C. Guaiacol-dependent peroxidase was followed at 470 nm (extinction coefficient = 26.6 mM^{-1} cm^{-1}), and when ferulic acid was used as a substrate, reactions were recorded at 310 nm (extinction coefficient = 16.3 mM^{-1} cm^{-1}).

ASC Determination

When indicated, ASC was determined in culture medium and soluble cytosolic fractions by a direct reading at 265 nm (extinction coefficient = 14.3 mM^{-1} cm^{-1}), followed by the addition of 300 milliunits of ascorbate oxidase (Sigma), to correct errors that were due to nonspecific absorbance.

Protein Determination

Protein was determined by the dye-binding method of Bradford (1976), using bovine γ-globulin as a standard.

Statistical Analysis

In all experiments mean values were compared using Student's *t* test. Statistical analysis of the root-growth kinetics under the different treatments were performed by analysis of covariance using a General Linear Methods procedure (SAS 6.04 software, SAS Institute, Cary, NC).

RESULTS

Root Growth Was Stimulated by ASC and GL, Its Biosynthetic Precursor

When onion bulbs were grown in the presence of ASC or GL, a significant stimulation ($P < 0.001$) of root lengths was observed. After 40-h treatments, onion roots that had been cultured in 1 mM ASC were 50% longer than the control roots, whereas 2 mM of GL increased root lengths by about 27% (Fig. 1). However, DHA, the oxidized form of ASC, was inhibitory, since roots reached only 60% of the lengths ($P < 0.0001$) observed in the control roots (Fig. 1).

Root Elongation Was Reversibly Inhibited by Lyc, an Inhibitor of ASC Biosynthesis

Lyc is a well known inhibitor of the last reaction, leading to the synthesis of ASC in plants (De Gara et al., 1994). When onions were treated with 50 μM Lyc for 40 h, roots grew to only about 28% ($P < 0.001$) of the lengths of the controls. The presence of 1 mM ASC or 2 mM GL did not prevent Lyc inhibition (Fig. 2). However, if Lyc was eliminated from the culture medium by washing after 15 h of treatment, root length increased again at a rate similar to that observed for control roots (not shown). Treatments with Lyc that exceeded 20 h resulted in an irreversible inhibition of root growth.

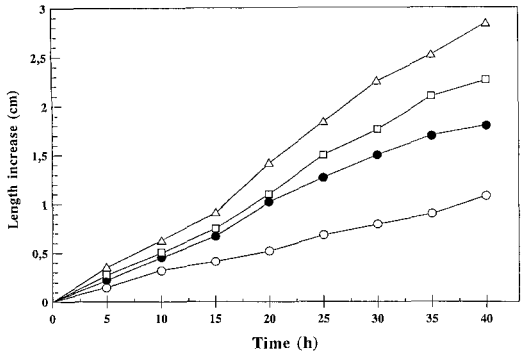

Figure 1. Effects of ASC, DHA, and GL on onion root growth. Onions grown in water for 3 d were transferred to medium containing 1 mM ASC (△), 1 mM DHA (○), 2 mM GL (□), or without addition (●, control). Root lengths were measured at the indicated times. Five bulbs were used for each treatment. Data included in Figures 1 to 3 represent the mean values of three independent experiments.

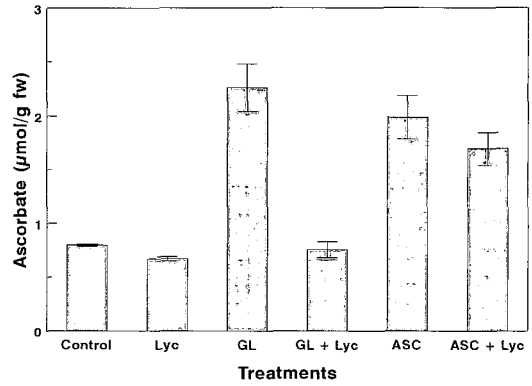

Figure 3. Effects of GL and Lyc on ASC concentrations in onion roots. Onion roots of 3 to 4 cm in length were grown for 15 h in water supplemented with 50 μM Lyc, 2 mM GL, or 1 mM ASC. The soluble cytosolic fraction was then obtained and ASC was determined as indicated in "Materials and Methods." The bars represent SE. fw, Fresh weight.

Intracellular Concentration of ASC Changed with the Composition of Culture Medium

Onion roots grown for 15 h in the different solutions were processed for the determination of ASC in soluble cytosolic fractions as indicated in "Materials and Methods." Results are indicated in Figure 3. Onion roots grown in water contained an ASC concentration of about 0.8 μmol/g fresh weight. When culture medium was supplemented with ASC or GL, ASC levels significantly increased to 149 and 183% versus control levels, respectively. However, Lyc reduced ASC levels to control values when added to GL-supplemented cultures; this inhibitory effect was less important in the case of ASC-supplemented medium. When Lyc was added to the control medium in the absence of ASC or GL, the ASC concentration was decreased 16% in the soluble fraction.

ASC Inhibited Peroxidase Activities in Vitro

Guaiacol peroxidase activity determined in the apoplastic fluid was inhibited in the presence of ASC (Fig. 4A). Ferulic-acid-dependent peroxidase followed inhibition kinetics very similar to that observed with guaiacol as the substrate (Fig. 4B). The inhibition time was proportional to the ASC concentration, and ASC inhibition ceased as soon as ASC was oxidized. In contrast, peroxidases isolated from cell-wall preparations responded to ASC in a different way, depending on the substrate that was used. With guaiacol, cell-wall peroxidase activity was also inhibited by

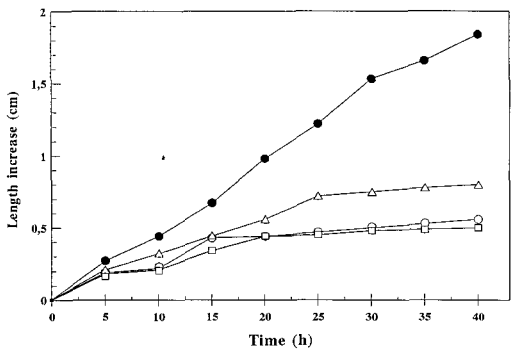

Figure 2. Effects of Lyc on onion root growth. Onions grown in water for 3 d were transferred to medium containing 50 μM Lyc (○), alone or in the presence of 1 mM ASC (△) or 2 mM GL (□). Experimental details and significance of data are as in Figure 1. Increases of root length in control onions are also shown (●).

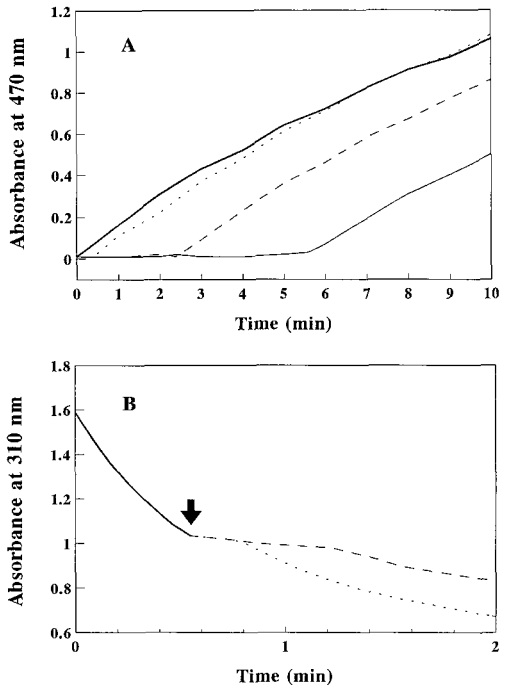

Figure 4. In vitro effects of ASC on guaiacol (A) and ferulic acid (B) peroxidase activities from the apoplastic fluid. Roots from onion bulbs grown in water for 5 d were excised and processed to obtain the apoplastic fluid. A, Guaiacol-dependent peroxidase was assayed in the presence of 10 (. . . .), 20 (- - - -), and 50 (—) μM ASC. B, Ferulic acid-dependent peroxidase was measured in the presence of 20 (. . .) and 50 (- - - -) μM ASC. Arrow denotes the ASC addition.

ASC, as long as ASC was present in the assay medium (Fig. 5A). However, cell-wall peroxidase activity assayed in the presence of ferulic acid was only partially inhibited by ASC, which was dependent on the ASC concentration (Fig. 5B). DHA did not affect peroxidase activities in either the apoplast or the cell wall (not shown).

ASC and GL Treatments Reduced Apoplastic and Cell-Wall Peroxidase Levels in Vivo

Preincubation of onion roots for 15 h in the presence of ASC decreased both guaiacol and ferulic acid peroxidase activities that were isolated from apoplastic fluid by about 25% (Table I). These activities were diminished by about 50% when cell-wall preparations were used as the source of enzymes (Table II). DHA preincubation enhanced peroxidase activities in both apoplastic fluid and the cell wall, with the exception of the peroxidase activity against ferulic acid that remained constant in cell-wall preparations (Tables I and II).

Lyc Increased Peroxidase Activities in Vivo

When onion bulbs were preincubated in the presence of Lyc, guaiacol- and ferulic-acid-dependent peroxidase activities in apoplastic fluid were significantly increased by about 15% or more. The presence of ASC with Lyc resulted in apoplastic peroxidase levels as in the control, although cell-wall-bound peroxidase activities were decreased by 12 to 23%. When preincubations were carried out in the presence of GL and Lyc, peroxidase activities were significantly enhanced (Tables I and II).

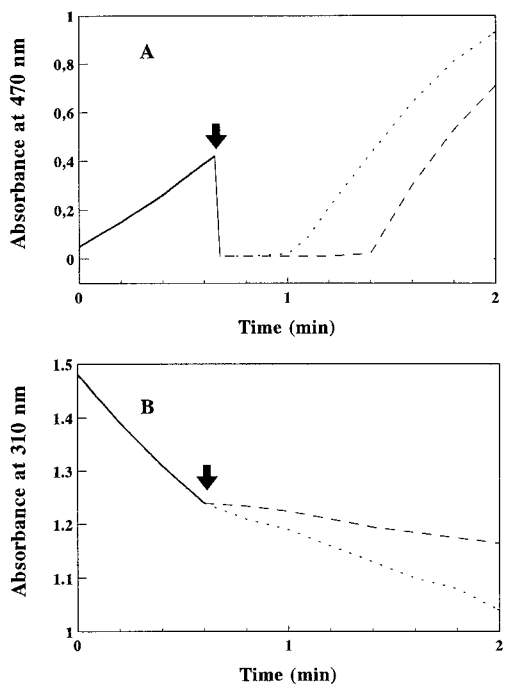

Figure 5. In vitro effects of ASC on cell-wall-bound guaiacol (A) and ferulic acid (B) peroxidase activities. Cell-wall fragments were obtained as indicated in "Materials and Methods" and peroxidase activities were assayed. Arrows indicate the addition of 20 (. . .) or 50 (- - - -) μM ASC.

DISCUSSION

Root growth is a complex process, involving cell proliferation, elongation, and differentation. ASC is a common compound in plants that may be physiologically involved in the regulation of cell-wall extensibility. Our group has published a number of observations that indicate that ASC and its free radical stimulate root elongation and accelerate the quiescence-proliferation transit in onion roots (Hidalgo et al., 1991; De Cabo et al., 1993; González-Reyes et al., 1994b), and similar results are shown in this paper. Onions grown in the presence of ASC had significantly longer roots than onions grown in control conditions; however, DHA inhibited cell elongation, supporting our previously reported data (González-Reyes et al., 1994a).

The stimulating effect of ASC on plant growth has been attributed to different mechanisms. Arrigoni's group has emphasized the role of ASC during cell division at the root meristem (see Arrigoni [1994] for a recent review), and in contrast, Navas's group has suggested that growth is stimulated on the basis of an NADH-AFR oxidoreductase linked to the plasma membrane (Navas, 1991; Córdoba and González-Reyes, 1994; Navas et al., 1994). Furthermore, Arrigoni (1994) has proposed that AFR formed from ASC also may be involved in the enlargement of the ER membranes and provacuole acidification, thus promoting cell expansion. Takahama and Oniki (1994) have suggested the possibility that ASC located at the cell wall might regulate elongation through the control of peroxidase-dependent oxidative polymerization or cross-link formation in cell walls. Apoplastic and cell-wall peroxidases are involved in the formation of phenolic cross-links that lead to the stiffening of the cell wall, so a negative correlation between peroxidase levels and the rate of cell expansion has been proposed (Fry, 1986; Zheng and Van Huystee, 1992; Lee and Lin, 1995; Sánchez et al., 1995).

ASC concentration in plants depends on several factors. For instance, the ASC/DHA ratio was highly modified during seed development in *Vicia faba* (Arrigoni et al., 1992), and the ASC concentration in the apoplastic fluid was responsive to hormone treatments (Takahama, 1994; Takahama and Oniki, 1994). Therefore, ASC concentration at the cytosol and at the apoplast seems to be modulated by developmental and environmental conditions.

The last step in ASC biosynthesis in plants is catalyzed by a dehydrogenase using GL as the substrate (Isherwood et al., 1954), apparently linked to the mitochondrial membrane in spinach (Mutsuda et al., 1995). GL is promptly converted to ASC in the cell (Arrigoni, 1994), and Lyc inhibits GL conversion to ASC (De Gara et al., 1994). Our results show that Lyc decreased the ASC concentration only 14% versus the control (Fig. 3). This percentage probably corresponds to the ASC that was synthesized after 15 h of incubation in buffered medium without additions. When onions were cultured in the presence of GL, symplastic ASC concentration increased about 3-fold over the control. Lyc inhibited the de novo synthesis of ASC from

Table I. *In vivo effect of ASC, GL, DHA, and Lyc preincubations on peroxidases isolated from the apoplastic fluid*

Onion bulbs were grown in culture medium supplemented with 1 mM ASC, 2 mM GL, 1 mM DHA, and/or 50 μM Lyc. After 15-h treatments, roots were cut off and washed twice in distilled H_2O. The apoplastic fluids were then extracted and guaiacol- and ferulic-acid-dependent peroxidase activities were measured. Data represent the means of four independent experiments.

Treatment	Peroxidase Activities			
	Guaiacol	Percent	Ferulic Acid	Percent
	(nmol min^{-1} mg^{-1} protein)			
Control	1154 ± 100	100	3691 ± 317	100
ASC	865 ± 66	75[a]	3119 ± 237	85[a]
DHA	1442 ± 147	125[b]	4614 ± 429	125[a]
GL	876 ± 75	76[a]	3504 ± 410	95
Lyc	1326 ± 128	115[b]	5021 ± 385	136[a]
ASC + Lyc	1140 ± 109	99	3566 ± 289	97
GL + Lyc	1477 ± 171	128[a]	4798 ± 350	130[a]

[a] $P < 0.01$ versus control. [b] $P < 0.05$ versus control.

GL, and when onions were grown in the presence of ASC, the intracellular pool of ASC was increased again. However, Lyc was able to diminish this increase by only 15%, corresponding to the value of net synthesis of ASC. Thus, the intracellular increase of ASC after ASC incubation might be attributed mainly to uptake mechanisms. In conclusion, GL and Lyc treatments may reproduce physiological changes of ASC concentration in roots. Our results show that GL mimicked ASC effects on the elongation rates of onion roots, whereas Lyc reversibly blocked root elongation. Although the inhibitory action of Lyc on the synthesis of Hyp-rich proteins (Arrigoni et al., 1977) and on the cell-cycle progression (Liso et al., 1984) was prevented by the simultaneous addition of ASC, its effect on root elongation was not prevented by ASC, indicating that Lyc inhibits root growth by mechanisms other than those depending on the inhibition of ASC biosynthesis.

Newly synthesized ASC may be partially transported to the apoplast (Rautenkranz et al., 1994). Transport of ASC from the cytoplasm to the apoplast has been observed by Castillo and Greppin (1988) in leaves of *Sedum album* and by Luwe et al. (1993) in spinach leaves subjected to ozone exposure. ASC is a common molecule in the apoplast (Castillo and Greeping, 1988; Polle et al., 1990; Takahama and Oniki, 1992; Luwe et al., 1993), and in this compartment ASC may regulate peroxidases involved in the cross-linking of cell-wall polymers. We have tried to measure ASC in the apoplast compartment. However, our efforts were unsuccessful because of the absence of spectrophotometrically detectable ASC in the apoplastic fluid obtained after vacuum-infiltration and centrifugation at 1500*g*. When centrifuge forces were enhanced, ASC was then detectable, but a significantly high activity of the cytosolic Glc-6-P dehydrogenase was observed as well, thus indicating that this ASC came from the cytosolic compartment. Since we used onions that were grown hydroponically, the possibility exists that small soluble molecules at the apoplast were equilibrated with the culture medium, and ASC was diluted and became undetectable using standard methods. However, peroxidases were recovered from the apoplastic fluid without any Glc-6-P dehydrogenase contamination, probably because of their higher molecular weight.

Our data show that ASC inhibited in vitro guaiacol- and ferulic acid-dependent peroxidases isolated from apoplastic fluid and the cell wall. Peroxidase activities measured by guaiacol in both compartments were completely abolished but released after the ASC that was present in the assay media was oxidized. Ferulic-acid-dependent peroxidase behaved as guaiacol peroxidase when apoplastic fluid

Table II. *In vivo effect of ASC, GL, DHA, and Lyc preincubations on cell-wall-bound peroxidases*

Onion bulbs were grown as described in Table I. After 15-h treatments, roots were cut off and washed twice in distilled H_2O. Cell walls were then isolated and guaiacol- and ferulic-acid-dependent peroxidase activities were measured. Data represent the means of four independent experiments.

Treatment	Peroxidase Activities			
	Guaiacol	Percent	Ferulic Acid	Percent
	(nmol min^{-1} mg^{-1} protein)			
Control	171 ± 13	100	137 ± 12	100
ASC	103 ± 10	60[a]	69 ± 9	50[a]
DHA	233 ± 22	136[b]	134 ± 18	98
GL	110 ± 11	64[a]	72 ± 10	53[a]
Lyc	189 ± 21	111	154 ± 13	112
ASC + Lyc	150 ± 14	88	105 ± 12	77[b]
GL + Lyc	186 ± 26	109	172 ± 16	126[b]

[a] $P < 0.01$ versus control. [b] $P < 0.05$ versus control.

was used as the source of the enzyme. However, this activity, measured at the cell-wall preparations, was partially inhibited by ASC in a dose-response manner. Takahama (1993a, 1993b) and Otter and Polle (1994) reported similar results for apoplastic and cell-wall-bound coniferyl alcohol-dependent peroxidase, showing the appearance of ASC as a competitive inhibitor of the cell-wall-bound peroxidases. Takahama emphasized this ASC inhibition pattern in relation to the physiological regulation of cell-wall peroxidases by apoplastic ASC during growth in plants. Our results and those reported by these authors emphasize the differences between the substrates used for peroxidase assays as well as the different peroxidase proteins.

Although apoplastic ASC was not detectable, the possibility that ASC or GL treatments stimulate root elongation by a transient increase in apoplastic ASC, resulting in the inhibition of the apoplastic/cell-wall peroxidases, cannot be excluded. Neither GL nor Lyc affected peroxidase activities in vitro, indicating that ASC is the inhibitor of peroxidases.

In vivo studies on peroxidase activities were carried out by modifying total ASC pools in the root. GL and ASC preincubations that increased ASC concentrations in soluble cytosolic fractions reduced guaiacol- and ferulic-acid-dependent peroxidases in both the apoplastic fluid and isolated cell walls. In contrast, Lyc treatments increased peroxidase activities 11 to 36%, although intracellular ASC concentrations were similar to those observed in control-grown roots. The simultaneous addition of Lyc and ASC did not affect guaiacol peroxidases, but decreased ferulic-acid peroxidases (12–23%). Additionally, Lyc plus GL increased all peroxidase activities (9–30%). Therefore, it seems then that the intracellular ASC levels modulate the peroxidases that are involved in cell expansion.

As explained above, these results might be attributed to the stationary changes in the ASC pools, thus modulating peroxidase activities as in vitro studies. However, assays of peroxidases after in vivo treatments were performed in preparations obtained from repeatedly washing roots, so assay medium did not include ASC. Thus, our data suggest that ASC might regulate peroxidase synthesis and/or secretion out of the cells. If so, the increased ASC synthesis during the first developmental stage in plants (Arrigoni et al., 1992) might be negatively correlated with the levels of the peroxidases that are specifically involved in the cross-linking reactions at the cell wall, hence plant elongation would be stimulated. In a similar way, ASC inhibited in vivo the cross-linking of soluble extensin by blocking the formation of isodityrosine in root slices of *Daucus carota* (Cooper and Varner, 1984), suggesting that extensin stiffens the wall during growth, thus reducing the rate of elongation (Cassab and Varner, 1988). Besides, ascorbic acid treatment abolished the blue-light, calcium-dependent inhibition of stem elongation in *Cucumis* (Shinkle and Jones, 1988).

In conclusion, our results strongly support the suggestion by Fry (1986) that peroxidase-driven reactions at the cell wall decrease its extensibility by increasing the cross-linkage reactions of several proteins and other polymers. ASC stimulates the growth rate by modulating in vivo peroxidase activities and/or the turnover of peroxidases involved in cell elongation, or even their secretion to the apoplast/cell-wall compartments. Plant peroxidase activities include a high number of proteins, and probably particular peroxidases are exclusively linked to growth control. Penel and Castillo (1991) associated anionic peroxidases to the cell-wall lignification and rigidification, whereas cationic peroxidases seem to play a role in the defensive mechanisms against oxidative stress. It has been reported that the enzyme activity of a particular fraction of cell-wall peroxidases decreased when cell-wall loosening was induced by an acid pH (Valero et al., 1991). Thus, future investigations may focus on whether ASC modulates particular peroxidases involved in cell-wall stiffening, and in identifying the specific physiological mechanism(s) by which ASC affects peroxidase-catalyzed reactions at the cell wall during cell expansion and root elongation.

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