Peroxidases In Tobacco Abscission Zone Tissue

II. TIME COURSE STUDIES OF PEROXIDASE ACTIVITY DURING ETHYLENE-INDUCED ABSCISSION

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ABSTRACT

Ethylene-induced abscission in flower pedicels of Nicotiana tabacum L. cv. Little Turkish causes a progressive increase in peroxidase activity during the first 4 hours of a 5-hour time course ethylene treatment period, with decrease in peroxidase activity occurring between 4 hours and 5 hours, when the supernatant extracts of abscission zone segments are tested spectrophotometrically for peroxidase activity, using guaiacol and hydrogen peroxide. Nonethylene-treated tissue has a much lower level of peroxidase activity over the same time course period. In ethylene-treated tissue the decline in break-strength correlates with the beginning of increase in peroxidase activity (3 hours). When the abscission zone area of the pedicel is further divided into proximal, abscission zone, and distal portions, respectively, the ethylene-treated tissue has the highest peroxidase activity in the abscission zone portion, with the maximum peak occurring at 4 hours and decreasing between 4 hours and 5 hours. Acrylamide gel electrophoresis of enzyme breis from ethylene-treated and nonethylene-treated plants reveals that no new peroxidase isozymes are formed in response to ethylene, indicating an increase in the amount of one or in both of the two already existing isozyme banding patterns. The measurement of protein in the proximal, abscission zone, and distal segments, over a 5-hour ethylene treatment period, indicates that it is being translocated in a distal to proximal direction in the abscission zone pedicel. The possible participatory role for peroxidase in ethylene-induced tobacco flower pedicel abscission are discussed.

Ethylene can stimulate peroxidase activity in petiole, stem, and leaf blade tissue of cotton plants (6, 12); in the pulvinus and separation layer of bean explants (3, 14); in etiolated peas (1); and in sweet potato root discs (3). It has been suggested that the distribution of peroxidase activity and IAA oxidase content can be correlated with the processes of natural abscission (10, 11, 13, 17). This study presents kinetic data on ethylene-induced peroxidase activity in the abscission zone of tobacco flower pedicels. A preliminary report on the results of these experiments was published earlier (4). Histochemical data at the electron microscope level, relating to these experiments, have also been reported (5).

MATERIALS AND METHODS

Tobacco plants (Nicotiana tabacum L. cv. Little Turkish) were grown with subsequent exposure or nonexposure (controls) to ethylene (5 μl/l) over a 5-hr time course period as previously described (22). Tissue segments containing the abscission zone were excised from the pedicels of the flowers at the beginning of the experiment and then at hourly intervals beginning 1 hr after the initiation of ethylene (5 μl/l) exposure (Fig. 1).

Groups of 10 segments (each segment 6 mm in length) from the flower pedicels were used for the spectrophotometric assay to determine peroxidase activity. A separate group of 10 segments was collected, and the 6-mm portion of the flower pedicel was further divided into distal (2 mm), abscission zone (2 mm), and proximal (2 mm) segments. Peroxidase activity in enzyme breis prepared from tissue segments was determined where the tissue had been previously exposed to ethylene for periods up to 5 hr.

After excision from the flower pedicel the tissue segments (6- and 2-mm sections) were weighed and immediately put into prechilled mortars at 4 C. The tissue samples were ground with a mortar and pestle at 4 C for 5 min in 4 ml of 0.01 M phosphate buffer, pH 6.0. The tissue homogenates were centrifuged for 10 min at 4 C at 2,000g to remove cellular debris. The supernatant fraction was used for the peroxidase assays (15).

Spectrophotometric determinations were measured at a wavelength of 460 nm, using a Beckman model DU spectrophotometer. The standard solution consisted of 1.3 ml of 0.5% guaiacol, 1.3 ml of 5 mM H2O2, plus 0.8 ml of distilled water (42). The experimental solution contained 1.3 ml of 0.05% guaiacol, 1.3 ml of 5 mM H2O2, plus 0.8 ml of enzyme supernatant prepared as described above (15). The enzyme supernatant was added at time zero, and the change in absorbance was noted at 5-sec intervals, over a period of 3 min. The linear portion of the curve was taken over the range between 70 and 150 sec, and the final relative peroxidase activity was expressed as units/g fresh weight of tissue (3, 15). The standard error was computed for each tissue sample (representing 10 separate determinations). Protein determinations were measured spectrophotometrically, using enzyme breis prepared as described above for the peroxidase assays, with bovine serum albumin being used as a standard (9). In Figures

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PEROXIDASES IN ABSCISSION ZONE

BREAK-STRENGTH

TIME (hrs)

Fig. 1. A representative portion of the tobacco flower pedicels selected for assays from ethylene-treated (5 μl/l) and nonethylene-treated plants. A 6-mm portion of the pedicel in the abscission zone region (arrows) was used in assays for peroxidase activity (Fig. 2). The 6-mm pedicel segment was divided into 2-mm sections designated as proximal, distal, and abscission zone portions, with each particular segment being assayed for peroxidase activity and protein content in ethylene-treated (5 μl/l) and nonethylene-treated plants (Figs. 2 to 8).

Fig. 2. A comparison of pedicel break-strength across the abscission zone (□, ▽) with peroxidase activity (○, △) over a 5-hr time course period of ethylene exposure (□) as compared to nonethylene-treated controls (▽). For acrylamide gel electrophoresis, segments of ethylene-treated and nonethylene-treated flower pedicel tissue were selected as described above, and the respective samples (20 segments each) were ground with mortar and pestle at 4 C in 1.0 ml of 0.8% NaCl and 0.1% NaNO₃, pH 5.9 (20). The separate homogenates were centrifuged at 4 C for 1 hr at 5,000g. The enzyme extract (0.5 ml) containing about 100 μg of protein (8) was applied to each polyacrylamide gel tube for electrophoretic separation (19, 20). A current of 5 mamp per column was applied in o-dianisidine reaction mixture to show the peroxidase isozyme bands (19, 20).

For break-strength determinations, flower pedicels were prepared as described above for control and ethylene-treated growth chamber experiments. Stems containing 10 or more flower pedicels were withdrawn from the control and ethylene treatment chambers at intervals of 2, 3, 3.5, 4, 4.5, and 5 hr. Each average measurement represents the break-strengths of 10 separate flower pedicels. Individual break-strength determinations were made with flower pedicels excised 5 mm proximal to the separation layer. The break-strength is defined as the amount of force, in grams, necessary to cause complete separation of the cell layers comprising the abscission zone (2, 4).

RESULTS

Peroxidase activity in the 6-mm pedicel segments increases after 3 hr of exposure to ethylene, with a perceptible increase...
beginning of the decrease in break-strength which is necessary to cause separation across the abscission zone (Fig. 2). The peroxidase activity reaches a maximum after 4 hr of ethylene treatment and the break-strength is progressively lower at this point (Fig. 2). Between 4 and 5 hr, peroxidase activity decreases while the break-strength continues to decrease to a relatively low level at 5 hr, at which time ethylene-induced abscission is occurring (Fig. 2).

A measurement of protein in the 2-mm distal, abscission zone, and proximal segments indicates that a relatively high level is present in the distal portion after 4 hr of ethylene exposure, followed by a decrease between 4 and 4.5 hr and a higher level at 5 hr when the pedicels are abscising (Figs. 6 to 8). The abscission zone portion, in ethylene-treated tissue, shows a relatively high level of protein at 4 hr, followed by a decrease between 4 and 4.5 hr with an increase between 4.5 and 5 hr to a value slightly larger than that observed at 4 hr (Fig. 6). The proximal segments show the highest level of protein after 4 hr of ethylene treatment.

Electrophoretic separation of enzyme breis from ethylene-treated (5 μl/l) abscission zone tissue (Fig. 3); distal (Fig. 4); and proximal (Fig. 5) tissue as compared to nonethylene-treated controls (▼).

Fig. 3-5. Five-hour time course study of peroxidase activity in ethylene-treated (5 μl/l, ▲) abscission zone tissue (Fig. 3); distal (Fig. 4); and proximal (Fig. 5) tissue as compared to nonethylene-treated controls (▼).

When the 6-mm flower pedicel segment is divided into 2-mm sections comprising proximal, abscission zone, and distal portions (Figs. 3 to 5) there is a much higher amount of peroxidase activity in the abscission zone segments after 4 hr of ethylene treatment, followed by a sharp decrease in activity between 4 and 5 hr in comparison to nonethylene-treated controls (Fig. 3). A smaller increase in peroxidase activity is also observed in extracts of tissue segments taken distally to the abscission zone from flowers treated with ethylene for 4 hr (Fig. 4). The proximal segments have the lowest levels of peroxidase activity over the 5-hr time course period regardless of whether the tissue is exposed to ethylene or to air without ethylene (Fig. 5).

Break-strengths (at the abscission zone) were determined at hourly intervals during the 5-hr period. Pedicels bend rather than break, during the first 3 hr of exposure to ethylene (Fig. 2). Break-strengths of the pedicels lead to breakage during the remaining portion of the 5-hr treatment period (Fig. 2). Nonethylene-treated control pedicels only “bend” over the 5-hr time course period (Fig. 2). The rise in peroxidase activity begins at 3 hr in ethylene-treated tissue and this parallels the occurrence of a decrease in break-strength which is required to cause separation across the abscission zone (Fig. 2). The peroxidase activity reaches a maximum after 4 hr of ethylene treatment and the break-strength is progressively lower at this point (Fig. 2). Between 4 and 5 hr, peroxidase activity decreases while the break-strength continues to decrease to a relatively low level at 5 hr, at which time ethylene-induced abscission is occurring (Fig. 2).

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treated and nonethylene-treated flower pedicels indicates that there are two major isozyme bands in the control and ethylene-treated gels. The increase in peroxidase activity that occurs after 3 hr of ethylene treatment may be associated with an increase in the width of the most anodic band after 3 and 5 hr of ethylene treatment (Fig. 9).

**DISCUSSION**

The fact that peroxidases might act on abscission via auxin destruction is apparent. However, the close correlation of increase in peroxidase activity and cell wall weakening, time-wise, suggests that a more direct action of peroxidase may be involved. Perhaps the increase in peroxidase activity is associated with changes in hydroxyproline-rich proteins which in turn affect the extensibility characteristics of the walls. Ridge and Osborne (16) have reported that ethylene can influence peroxidases and hydroxyproline-rich protein in the cell walls of pea shoots. Hydroxyproline-rich proteins have been implicated in cell wall extension (7).

The marked increase in peroxidase activity that occurs beginning at 3 hr after treatment of tissue with ethylene (Figs. 3 to 5) may be due to: (a) de novo synthesis of new peroxidase isozymes; (b) an increase in the mobilization of previously inactive peroxidases that become activated by the exogenously applied ethylene; (c) a translation of existing mRNA; or (d) treatment of the tissue with ethylene which might alter the binding of peroxidases to wall or other cellular components. With respect to the formation of new peroxidase isozymes, the electrophoretic data indicate that no new isozymes are present in breis of tissue exposed to ethylene. Other researchers have reported as many as 33 bands of peroxidase activity from seedling roots of tobacco (20). It is conceivable that additional peroxidase isozymes are present in the abscission layer of the tobacco flower pedicel. These isozymes may have remained with the cell debris or they may not have been detected because of the interference of phenolic substances. Possibly the number of peroxidase isozymes present in the specialized cells of the abscission layer is simply fewer. The possibility that de novo synthesis of peroxidase may occur from existing mRNA is suggested by the literature (21, 22). Ethylene treatment of tobacco flowers results in an increase in rough endoplasmic reticulum in the abscission cells (21). Since there is no electrophoretic evidence for the appearance of new isozymes the increased peroxidase activity may be due to enhanced translation of existing mRNA, in view of recent evidence (22, 23).

In experiments where cycloheximide or actinomycin D was applied along with ethylene, the following results were observed (23). First, treatment of the abscission cells with actinomycin D did not prevent abscission nor did the inhibitor prevent the increase in rough endoplasmic reticulum. However, treatment of the tissue with cycloheximide did lead to a prevention of abscission as well as to no increase in rough endoplasmic reticulum (23). In a recent study utilizing histochemical techniques at the electron microscope level, an increased localization of peroxidases was observed in the walls of abscission cells (of tobacco flowers) exposed to ethylene (5).

In an earlier report (18), it was shown that protein mobilization occurs from the distal region of the abscission zone to the proximal region, in bean explants. In the studies of protein levels of pedicel tissue reported here, variations in levels were observed. These variations persisted despite the many attempts to modify the extraction techniques. However, ethylene treatment always resulted in a high level of protein present in the proximal tissue at 4 hr, the same time that peroxidase activity was high in the abscission layer (Figs. 3 to 8). Peroxidase activity always remained low in the proximal region. If peroxidases were represented among the proteins which may move between these regions, they must have been present in an inactive form outside the abscission layer.

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**LITERATURE CITED**


