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Peroxidase Activity in the Abscission Zone of Bean Leaves during Abscission[1]

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ABSTRACT

Peroxidase activity and localization in the abscission zone of bean leaves were studied histochemically and by gel electrophoresis. Deblading of bean leaves resulted in an increase in peroxidase activity in the abscission zone 2 to 4 days after deblading with highest activity just prior to separation. In debladed plants, the cell division in six to eight layers of cells preceded separation. An ethylene treatment (8 microliters per liter) induced separation of debladed petioles in approximately 24 hours and of intact plants in 36 to 48 hours. Ethylene treatment produced similar results in both debladed and intact plants. In ethylene-treated plants, whether debladed or not, enzyme localization was restricted to only two to three layers of cells with no cell division apparent prior to separation. Infrequent cell divisions were observed after treatment with 2-chloroethylphosphonic acid (1000 micrograms per liter) (Ethephon); however, other changes were similar to those observed with ethylene. Deblading and ethylene treatment resulted in changes in the six peroxidase isozymes observed in the abscission zone. Only four were observed in samples collected 2 centimeters below the abscission zone. Peroxidase bands IV and V increased significantly in debladed and ethylene-treated plants and peroxidase VI decreased only in debladed plants. The changes in peroxidase activity were invariably observed prior to separation in all treatments.

Peroxidase has been implicated in a number of diverse phenomena observed in plants (29, 31, 34, 35) including the synthesis of ethylene (21, 38). Similarly, conflicting reports (10, 18) indicate that ethylene synthesis is not regulated by peroxidase. The localized increase in protein and RNA synthesis necessary for separation to occur in the abscission zone of *Phaseolus vulgaris* is increased by ethylene (2) and has been associated with an increase in cellulase activity (1, 15, 26).

The effect of ethylene on plant enzyme systems such as peroxidase has been well documented (11, 12, 16, 31); for example, peroxidase in sweet potato slices increased 100-fold after exposure to low ethylene concentrations (34). These changes can be blocked by cycloheximide or actinomycin D; therefore, it seems likely that they are dependent upon the synthesis of new protein (2-4, 11). This study describes the localization of peroxidase, the qualitative and quantative changes in peroxidase, and the peroxidase isoenzyme patterns in the abscission zone of bean. Observations on the effect of ethylene and Ethephon on the same peroxidase parameters are given.

MATERIALS AND METHODS

Plant Culture. Bean seeds (*Phaseolus vulgaris* L. cv Contender) were germinated in vermiculite at 1000 ft-c of fluorescent light (16 hr day) at 21 ± 1 C and watered with nutrient solution (14). Seedlings were utilized for abscission studies 8 to 10 days after germination.

Initiation of Abscission. Abscission of the primary leaf petiole at the lower pulvinus was induced by deblading the petiole 1 cm from the stem or by applying ethylene or 2-chloroethylphosphonic acid to the intact plant or leaf. Lanolin paste was applied when the petiole was cut, to prevent desiccation. Both debladed and intact plants were treated with 8 μl/l ethylene by injecting the gas into a bell jar (23). Similarly, some debladed and intact plants were sprayed to the drip point with 500 to 1000 μg/l of Ethephon.[2]

Peroxidase Localization. Fresh, free-hand longitudinal sections were made of the abscission zone, a portion of stem, and the lower pulvinus. Localization of peroxidase in sections of debladed, ethylene-, and Ethephon-treated plants at various times (Figs. 1-4) was studied until separation was complete. Reagents for the localization of peroxidase (8, 29) were: 1% H_2O_2 and a 70% ethanol solution of 0.1 M benzidine. The peroxidase reaction was carried out on a glass slide. A drop of each reagent was placed in contact with the fresh tissue slice. The sections were incubated for 1 to 2 min, the resulting bubbles were removed by rinsing twice with distilled water, and color photographs were taken immediately to record enzyme localization. Potassium cyanide (0.1 M) was added to the reaction mixture to serve as a control. All black and white photographs were reproduced from color photographs.

Peroxidase Activity Estimates and Electrophoresis. One to 2 g of tissue from the abscission zone of 15 to 20 plants were ground in a mortar and pestle, with 5 ml 0.1 M Hepes buffer (pH 7.3-7.4) with 0.1 mM dithiothreitol and 0.5 to 1 g of insoluble polyvinylpyrrolidine, and strained through an acetate cloth. The extract was centrifuged at 20,000*g* for 30 min (22). Peroxidase activity of the supernatant (0.1 ml) was determined

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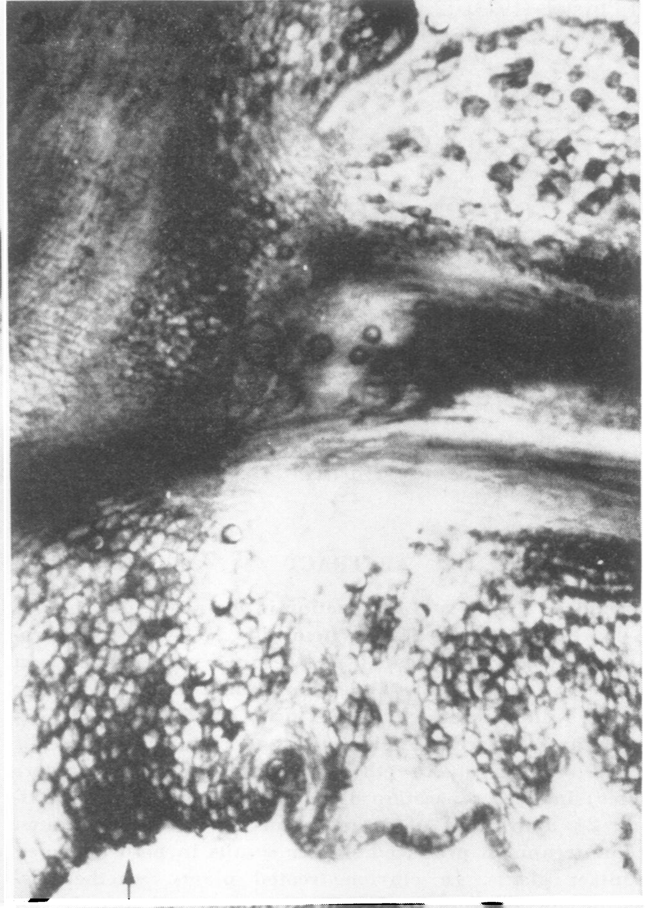
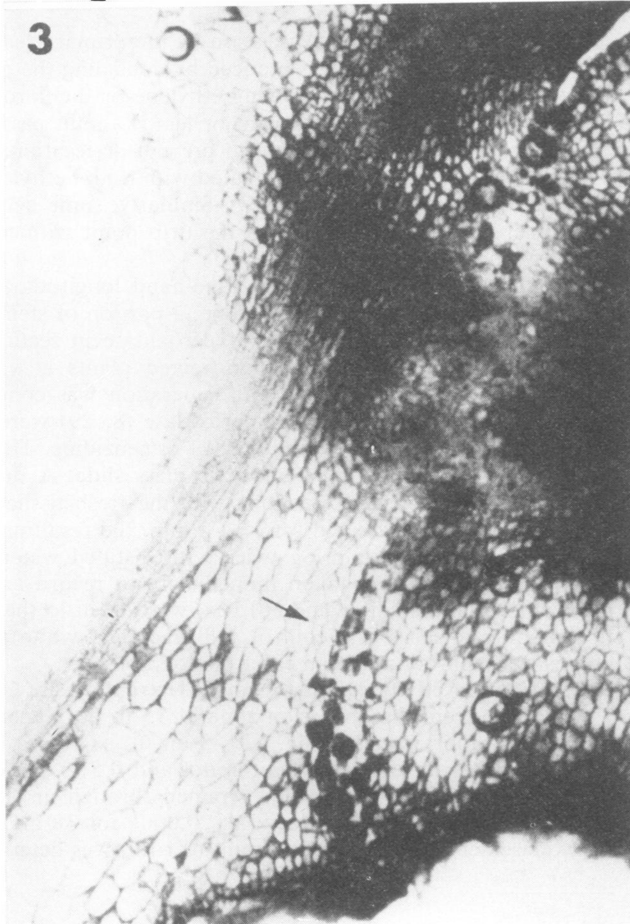
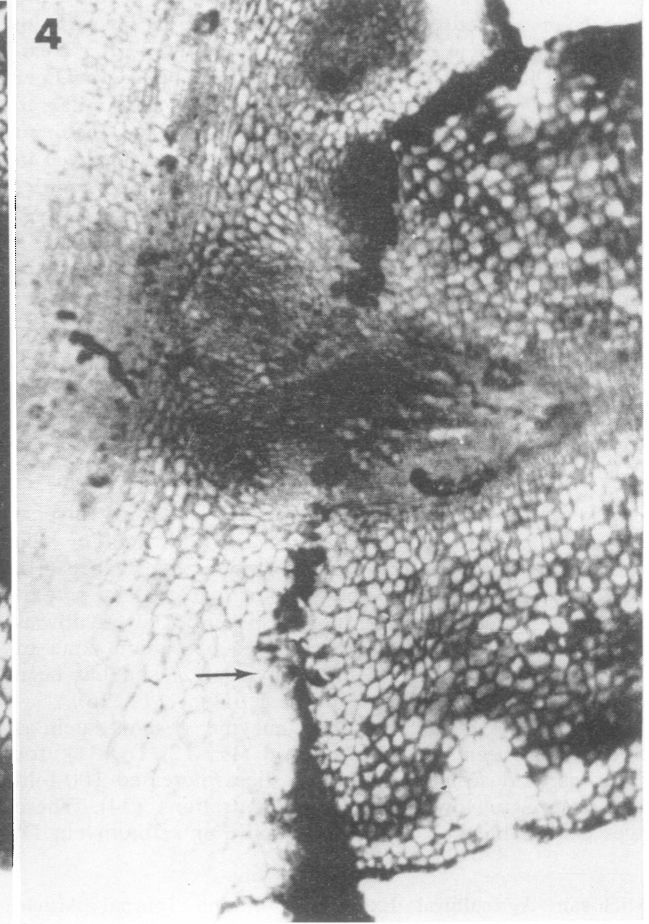
[2] Abbreviation: Ethephon: 2-chloroethylphosphonic acid.







FIGS. 1–4.

based on the oxidation of *p*-phenylenediamine at 485 nm (36). Controls included H_2O_2-deficient medium and heat-inactivated extract (100 C for 5 min). Electrophoretic separation of the supernatant proteins on polyacrylamide gel was carried out according to Davis (7). Enzyme extract (0.1 ml) containing about 200 μg of protein as determine by the Lowry method (20) was applied to each gel. A current of 2 ma per column was applied in tris-glycine buffer at pH 8.3. The gels were stained for 20 min in a benzidine reaction mixture (36) and photographed to show the peroxidase isoenzymes.

RESULTS

An increase in peroxidase activity was observed during abscission layer formation. Peroxidase localization is shown in the lower pulvinus (Fig. 1) and within the abscission layer (arrow) 3 days after deblading. Three to 4 days after deblading, plants had developed an abscission layer, and petiole separation occurred by 6 days. Cell division in 6 to 8 layers of cells in the abscission zone preceded separation in all debladed plants without ethylene treatment (Fig. 2). Foliar application of Ethephon induced abscission in 48 to 72 hr and increased peroxidase activity in three to four layers of cells (Fig. 3) in the abscission zone. Ethylene treatment of debladed and intact plants induced separation in 24 to 48 hr with peroxidase changes occurring most rapidly in the former. Ethylene increased peroxidase activity in two to three layers of cells in the abscission zone (Fig. 4). Cell division did not precede separation in intact plants treated with ethylene, confirming the work of Bednarz (5). This is not to imply that endogenous ethylene prevents cell division in natural leaf abscission. The concentration of ethylene used in this study was approximately 8-fold higher than endogenous levels. This concentration may have inhibited cell division but accelerated cell separation. This possibility is not inconsistent with the interpretation that cell division it not essential for abscission in bean explants.

Peroxidase activities from debladed and intact plants with and without ethylene treatment are illustrated in Figure 5. There was a significant increase in peroxidase activity in debladed and ethylene-treated intact plants during abscission layer development. This substantiates our *in vivo* observations (Figs. 1–4). Deblading and ethylene treatments also resulted in a change of isozyme patterns, as shown in Figures 6 and 7. Six peroxidase isozymes were observed in the extract from the abscission zone of control and debladed plants before formation of the abscission layer (Fig. 6). During abscission layer development, peroxidases IV and V showed a significant increase, whereas peroxidase VI decreased (Fig. 6). However, the overall enzyme activity increased (Fig. 5) both during and after abscission layer formation.

When ethylene was applied to intact bean plants, there was also a marked increase in total peroxidase. However, not all isoenzymes increased, as shown in Figure 7. Major increases

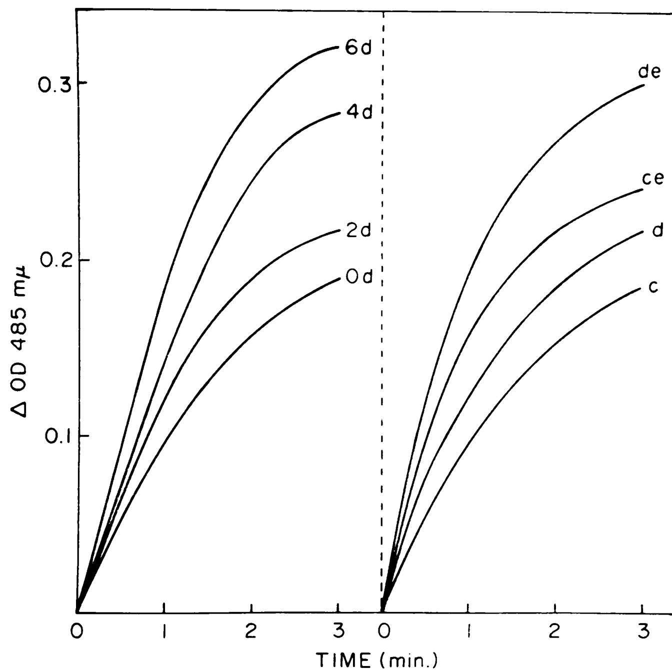


FIG. 5. Peroxidase activity of bean leaf abscission zones based on the oxidation of *p*-phenylenediamine. 0 to 6d: days after deblading; c: control; d: debladed; ce: control with 8 μl/l ethylene; de: debladed with 8 μl/l of ethylene.

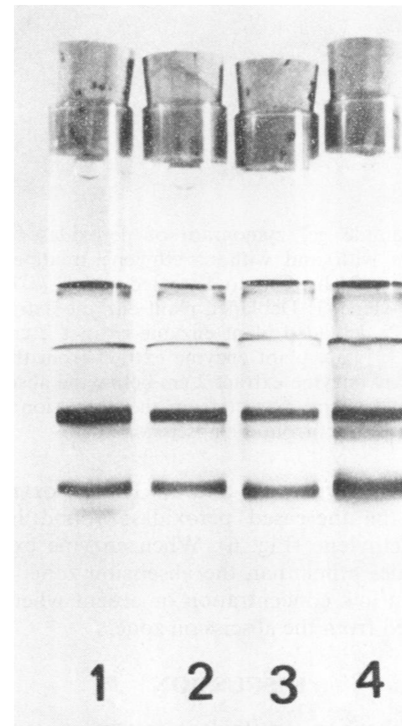


FIG. 6. Acrylamide gel zymogram of peroxidase from the abscission zones of debladed and intact plants. 1: Intact (control); 2: 48 hr after deblading; 3: 96 hr after deblading; 4: 144 hr after deblading.

FIG. 1. Localization of peroxidase (dark stain) in the lower pulvinus of bean leaves approximately three days after deblading. × 14. Arrows identify abscission layer or potential abscission layer.

FIG. 2. Abscission zone of bean leaf showing cell division (protective layer, arrow), convolutions in the epidermal tissue and the localization of peroxidase approximately three days after deblading. × 28

FIG. 3. Peroxidase localization in the abscission zone of Ethephon (1000 μg/l) sprayed leaf. Enzyme determination approximately 48 hr after treatment. Occasional cell division (arrow). × 28

FIG. 4. Peroxidase localization in the abscission zone of ethylene (8 μl/l for 24 hr) treated plant. Enzyme determination approximately 12 hr after the ethylene treatment. No cell divisions were evident. × 28

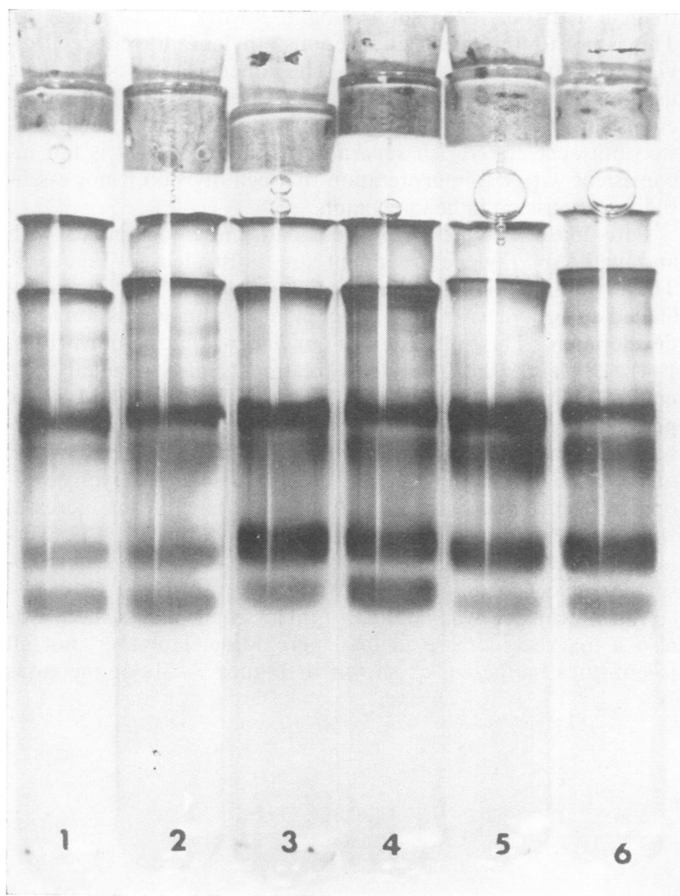


FIG. 7. Acrylamide gel zymogram of peroxidase of debladed and intact plants with and without ethylene treatment. Samples were collected after 24 hr. One to 4 treated with 8 μl/l ethylene, 5 and 6 without ethylene. 1: Debladed plant enzyme extract from the abscission zone; 2: debladed plant enzyme extract 2 cm below the abscission zone; 3: intact plant enzyme extract from the abscission zone; 4: intact plant enzyme extract 2 cm below the abscission zone; 5: debladed plant enzyme extract from the abscission zone; 6: intact plant enzyme extract from the abscission zone.

are evident in isoenzymes IV and V. These isozyme changes correspond to the increased peroxidase found in debladed plants without ethylene (Fig. 6). When enzyme extracts were taken from tissues other than the abscission zone, isoenzymes I and II were in low concentration or absent when compared to these extracted from the abscission zone.

DISCUSSION

Deblading, Ethephon, or ethylene treatments increased peroxidase activity in the pulvinus and in particular the separation layer of bean plants during abscission. By either deblading or treatment with ethylene the petiole, abscission layer, or perhaps both undergo an induction period (9, 19, 30, 32). Abscission is known to be an oxidative process (23, 33), and it has been shown that respiration is involved in this process (12, 27). Both the *in vivo* (Figs. 1–4) and *in vitro* (Figs. 5–7) studies reported here show changes in peroxidase activity that are specific to the abscission zone. The anatomical and histochemical changes which precede leaf fall are quite similar in intact and debladed plants (37), although abscission is greatly accelerated in the latter.

This work coupled with our earlier work has shown that deblading and ethylene treatments increase peroxidase, dehydrogenase, and acid phosphatase activities in the abscission zones of bean leaves (27), and similar changes were observed in mature cherry fruits (28). These and other reports (9, 15, 19, 27, 28) confirm a localized increase in respiration and an acceleration of cellular senescence. The increase in peroxidase activity in the pulvinar tissue suggests that it may have some control over the changes that occur in the abscission layer. Furthermore, the pulvinar tissue of bean explants is the principal site of ethylene production (17) and ethylene responsiveness (6, 24) and also possesses a reduced capacity to transport auxin (13). Increased peroxidase activity may affect ethylene synthesis (21, 38), the oxidation of IAA (13, 25, 35), or other senescence phenomena.

Cell division prior to separation is a common occurrence in intact and debladed plants. Lack of cell division in the abscission zone of ethylene-treated plants and only occasional cell division in Ethephon-treated plants show variation in abscission pattern. This variation may be due to different actual concentrations of ethylene or a difference in mode of action between Ethephon and ethylene. Bednarz (5) recently found that plants treated with ethylene do not form the protective layer prior to or in conjunction with separation layer formation. Lack of ethylene-induced cell division associated with an enhanced separation confirm that cell division is not a prerequisite for separation in the lower pulvinus of the bean. Formation of the protective layer in debladed and naturally abscising plants serves to prevent desiccation from the stem scar. No cell division during ethylene treatment demonstrates clearly that the role of peroxidase in abscission is confined to the formation of the separation layer and is not involved in protective layer formation. This is consistent with the observation that peroxidase is closely related to senescence phenomena. When bean plants were treated with ethylene or Ethephon, there was a localized increase in peroxidase activity in the separation layer and not in adjacent tissue.

Auxin is known to be effective in delaying the abscission layer formation. After induction the same concentration of auxin accelerates the abscission process (32). Later it was shown that ethylene did not influence abscission until stage II (4). The data reported here demonstrate that peroxidase is associated with the development of the separation layer but not the protective layer. Therefore, it is logical to place the action of peroxidase in stage II. Stage II is primarily a process of senescence in the abscission zone; correspondingly, peroxidase would be a predominant enzyme. The results suggest that an increase in peroxidase activity is closely tied to the development of the abscission layer. Its exact role in the abscission process remains to be elucidated.

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