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Immunocytochemical Localization and Time Course of Appearance of an Anionic Peroxidase Associated with Suberization in Wound-Healing Potato Tuber Tissue[1]

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ABSTRACT

Thin sections of wound-healing potato tuber tissue were stained with rabbit antibody prepared against a suberization-associated anionic peroxidase and then stained with a goat anti-rabbit antibody-fluorescein conjugate. When these sections were examined with an epiilluminating fluorescence microscope, bright green fluorescent linear deposits were observed on the inner side of cell walls in the periderm layer. Initial deposits which were often not contiguous throughout the wall were first observed in some cells after 3 days of wound-healing and subsequently these layers became more pronounced so that all 6 day old periderm cells had green fluorescent layers on their inner walls. This fluorescence was not present in the walls of parenchyma cells or in the walls of periderm cells treated with preimmune serum and anti-rabbit IgG-FITC conjugate. Thin sections of wound-healing potato tissue which were stained with anti-peroxidase antibody and a goat anti-rabbit antibody-rhodamine conjugate exhibited a similar time course of development with a bright reddish-orange fluorescent layer observed on the inside wall of periderm cells. The production of this suberization-associated anionic peroxidase in wound-healing tissue was also demonstrated by an immunobinding dot blot assay which showed that the largest increase in the enzyme level occurred between 4 and 6 days of wound-healing. The present results support the hypothesis that this anionic peroxidase is involved in the deposition of the aromatic polymeric domain of suberin.

Suberized periderm formation appears to be the common response to wounding of any plant organ irrespective of the natural protective layer of the organ (19, 21). Suberization results in the development of a diffusion barrier and thus seals off the wound from water loss (21). Suberin is a polymer most probably composed of aliphatic and aromatic domains (19, 20). The aromatic domain is thought to be somewhat similar to lignin and it was proposed that the polymerization of the aromatic monomers of suberin involves an isoperoxidase (19) in a manner similar to that previoulsy suggested for lignin biosynthesis (13, 14).

The biochemical processes of suberization have been examined in detail in wound-healing slices of potato tuber (2, 8, 21). In this tissue the development of diffusion resistance, the deposition of aliphatic and aromatic monomers into insoluble polymer, the production of suberin-associated waxes, and the induction of enzymes involved with suberization all follow a similar time course with detectable production beginning approximately 2 d after wounding and increasing to reach a maximum after 6 to 8 d (1, 6, 8, 21, 30). An anionic isoperoxidase was also shown to have the same time course of appearance in wound-healing potato tuber and to be localized in the wound periderm (4). Induction of suberization by ABA in potato tissue culture corresponded to the appearance of an anionic peroxidase (7) which was shown to be immunologically similar, if not identical, to the peroxidase from wound-healing slices (11). If this peroxidase is induced for the synthesis of suberin in the wound-healing tissue this enzyme should appear at the appropriate time during the wound healing process and it should be localized in the inside portion of the cell wall. In this paper we use indirect immunofluorescence microscopy to demonstrate the time course of appearance of this suberization-associated anionic peroxidase in wound-healing slices of potato and to determine its location within the walls of suberizing cells.

MATERIALS AND METHODS

Chemicals. [^{125}I]Protein A was purchased from New England Nuclear Corp. Anti-rabbit IgG-FITC conjugate, anti-rabbit IgG-TRITC conjugate, acridine orange, BSA, guaiacol, rhodamine B, and Triton X-100 were purchased from Sigma Chemical Company. White Rose potatoes and Carnation nonfat dry milk were purchased from a local grocery store. All other chemicals were of reagent grade.

Wound-Healing of Potatoes. Washed tubers of *Solanum tuberosum* cv White Rose were soaked for 30 min in 1% (w/v) hypochlorite solution and then thoroughly washed with water. The natural periderm was removed and 3-mm thick slices were cut from the tuber. Slices were soaked in water for 10 min, blotted dry, and stored in the dark at 20°C on rubberized mesh in gallon jars through which water-saturated air was passed as described in Kolattukudy and Dean (21). Slices were kept in this manner for 0 to 6 d and then used immediately for sectioning.

Tissue Preparation. Tissue representing all stages of wound-healing were prepared at the same time. Small tissue pieces (~2 × 4 mm with the wound surface being 2 mm^2) were cut from the potato slices with razor blades and immersed in 2.5% (w/v) paraformaldehyde in 50 mM phosphate buffer (pH 7.2). The tissue was chemically fixed for 4 h at 4°C, rinsed with 10 mM K-phosphate, pH 7.6. for 2 h (4 changes) at 4°C, dehydrated with ethanol and infiltrated with and embedded in L. R. White acrylic resin. Serial sections, 1 μm thick, were cut with glass knives and dried onto gelatin coated slides. Periderm location, development, and preservation in each tissue block sampled were monitored by light microscopic examination of crystal violet stained sections.

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Immunolabeling. Slides were pretreated in Copland jars for 2 h with 1% Carnation nonfat dry milk in 10 mM K-phosphate (18), pH 7.6, at 5°C and then incubated overnight (~16 h) at 5°C in a 1:500 dilution (v/v) of anionic peroxidase antiserum (11) in 10 mM K-phosphate, pH 7.6, containing 0.25% BSA. Slides were rinsed thoroughly with phosphate buffer (10 × 10 ml) containing 0.25% BSA, pretreated for 2 h at 5°C with 1% Carnation nonfat dry milk in phosphate buffer, and then incubated with a 1:1000 dilution of either anti-rabbit IgG-fluorescein conjugate or anti-rabbit IgG-rhodamine conjugate in phosphate buffer containing 0.25% BSA for 2 h at room temperature. Slides were then rinsed thoroughly with phosphate buffer containing 0.25% BSA. Some mounted sections were stained with 0.01% acridine orange for 4 min at room temperature and rinsed thoroughly with water. Other sections were treated with 0.001% rhodamine B in phosphate buffer with 0.25% BSA at room temperature for 2 h and rinsed thoroughly with water.

Microscopy. Sections mounted on glass slides were immersed in 1:1 mixture of glycerol and phosphate buffer under a coverslip and viewed with a Labolux II epifluorescence microscope (Leitz) equipped with a 50 W mercury vapor lamp operated at a power of 12 V. When sections were viewed for fluorescein fluorescence, autofluorescence and acridine orange staining a Leitz N2 filter was utilized (450 nm excitation light and a 490 nm barrier filter). When sections were viewed for rhodamine fluorescence a Leitz I 2/3 filter was utilized (530 nm excitation light and a 560 nm barrier filter). Color exposures were made with an Olympus C35AD camera coupled to an Olympus exposure control unit with Kodak Ektachrome ASA 400 print film. Exposure time for all micrographs of fluorescein fluorescence, autofluorescence, acridine orange staining and rhodamine fluorescence were 40, 120, 5, and 60 s, respectively. Prints were prepared commercially. These sections were also photographed using Kodak Technical Pan and phase contrast optics (Leitz).

Peroxidase Assays. Periderm layers were manually removed from potato slices which had wound-healed for periods of time ranging from 0 through 6 d. Acetone powder extracts were prepared for samples collected at 24 h intervals as described previously (11). These extracts were dialyzed against 10 mM potassium acetate, pH 6.0, and were assayed by adding 0.01 ml of enzyme extract to 2.0 ml of 0.1% guaiacol and 0.03% H_2O_2 in 50 mM potassium acetate, pH 6.0, and measuring the increase in *A* at 470 nm. Aliquots (0.2 ml) of the acetone powder extracts were applied to nitrocellulose membranes and assayed for the anionic isoperoxidase by an immunobinding assay (16) utilizing a 1:100 dilution of anionic peroxidase antiserum and [^{125}I]-Protein A (200,000 cpm/ml). Samples were counted in an Isodata 20/20 gamma counter.

RESULTS

Indirect Fluorescein Immunofluorescence Localization of Anionic Peroxidase in Wound-Healing Potato Tuber Slices. Potato tuber slices were allowed to wound-heal for various periods up to 6 d at 20°C under an atmosphere of water-saturated air. Thin sections of these slices were stained with rabbit antibody prepared against purified anionic peroxidase, and subsequently with goat anti-rabbit IgG-fluorescein conjugate and viewed with an epifluorescence microscope. Sections of freshly cut potato slices exhibited very little fluorescence (Fig. 1). There was some weak fluorescence in the sheath (envelope and stroma), encircling some of the starch grains. Light microscopy showed the starch grains to be very prevalent while fluorescence microscopy indicated approximately 10% of these grains had detectable anionic peroxidase in their sheaths. Tissue slices which had wound-healed for 1 or 2 d also exhibited fluorescence primarily in the stromal sheath of starch grains (Fig. 2). After wound-healing for 3 d the cells closest to the wound surface had undergone dramatic changes with yellow autofluorescent deposits seen as globular material in the vacuole and as linear arrays along the cell wall of some of the cells adjacent to the wound surface. Some cells in this region (1–3 cells from wound surface) also exhibited green fluorescein fluorescence in the inside of the cell wall (Fig. 3). Such fluorescence, indicating the presence of the anionic peroxidase on the wall, was seen in approximately 10% of the cell walls in the first two or three layers of cells adjacent to the wound surface of tissue which had been wound-healed for 3 d. Frequently the fluorescence appeared as a dotted line along the wall suggesting that the anionic peroxidase deposition was in its initial stages (Fig. 3). This peroxidase-specific fluorescence was only seen in the first three layers of cells adjacent to the wound surface which we designate as the periderm layer.

Cells in this periderm layer from tissue which was wound-healed for 4 d had very little autofluorescence but at this stage strong green fluorescence indicating the presence of anionic

FIGS. 1–6. Fluorescence micrographs of periderm cells of wound-healed potato tissue stained first with rabbit antibodies prepared against anionic peroxidase and then with goat anti-rabbit IgG-fluorescein conjugate.

FIG. 1. Freshly cut potato tuber slice showing no fluorescence in cell walls. Some starch grains showed slight green fluorescence in the sheaths (×500).

FIG. 2. Tissue wound-healed for 2 d revealing no fluorescence in cell walls (×500).

FIG. 3. Tissue wound-healed for 3 d. Green fluorescence in cell walls appears as dotted lines (arrowheads) (×500).

FIG. 4. Tissue wound-healed for 4 d with continuous region of green fluorescence in cell wall (×500).

FIG. 5. Tissue wound-healed for 5 d with long region of green fluorescence clearly located on inner side of periderm cell wall. (×500).

FIG. 6. Bright green fluorescent layer on the inside of cell wall in the periderm of tissue wound-healed for 6 d (arrowheads) with yellow-orange autofluorescence observed as a linear array on the inside of the wall and also as globular deposits in the cytoplasm (×500).

FIG. 7. Fluorescence micrograph of potato tuber tissue wound-healed for 6 d stained first with preimmune rabbit serum and then with goat anti-rabbit IgG-fluorescein conjugate. No green fluorescence is observed but autofluorescence of the wall (arrowhead) can be seen.

FIG. 8. Green fluorescence on inner side of long periderm wall and along a newly formed wall on the right side of the figure (arrowheads); potato tissue wound-healed for 6 d. Cell is the same as that shown in Figure 7 (×800).

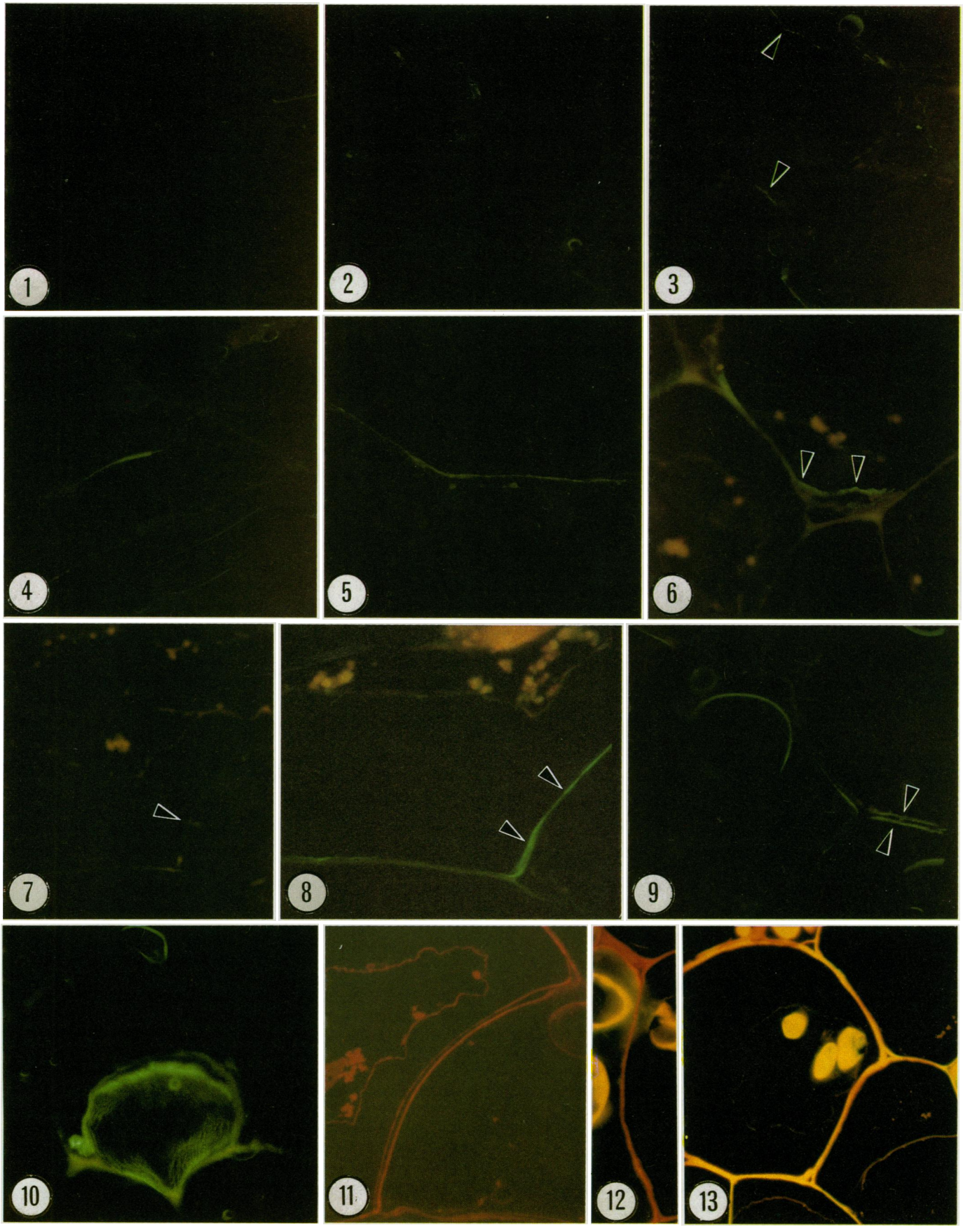
FIG. 9. Green fluorescence seen on both inner sides of a potato periderm cell wall (arrowheads) and on the inner wall of an adjacent cell to the left; tissue wound-healed for 6 d (×500).

FIG. 10. Potato tissue wound-healed for 5 d with outer cell wall cut tangentially revealing microfibrils of wall and bright green fluorescence. Yellow autofluorescence can be seen in background in inner layer of the wall (×500).

FIG. 11. Fluorescence micrograph of potato tissue wound-healed for 6 d which was stained first with anionic peroxidase antibody and then with goat anti-rabbit IgG-rhodamine conjugate; bright orange-red fluorescence exhibited on both inner sides of a periderm cell wall (×500).

FIG. 12. Fluorescence micrograph of freshly cut potato tissue stained with acridine orange. Cell walls exhibit bright orange fluorescence (×500).

FIG. 13. Fluorescence micrograph of potato tissue wound-healed for 6 d and stained with acridine orange. Periderm cell walls show bright yellow fluorescence (×500).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peroxidase was seen in portions of the wall of approximately half of these cells (Fig. 4). The cells at this stage of wound-healing (d 4) had noticeably fewer starch grains; the starch was probably utilized as a source of energy for the wound-healing process (3). Periderm cells in tissue which had been wound-healed for 5 d had longer stretches of green fluorescent walls (Fig. 5). This fluorescence was seen in the inside of the cell wall indicating the localization of the anionic peroxidase (Fig. 5).

In the periderm cells of potato tissue which had been wound-healed for 6 d green fluorescence indicating the presence of anionic peroxidase was seen in the walls of almost all (>90%) cells. Such fluorescence was frequently seen very clearly in the inside wall of a cell at regions where 3 to 4 cells came closely together (Fig. 6). Yellow and orange autofluorescent layers were usually seen in interior regions of these junction walls indicating the presence of phenolic materials. Globular autofluorescent deposits, presumably phenolic components, were also prevalent in the vacuoles of cells in the periderm of tissue which had been wound-healed for 6 d (Fig. 6).

Thin sections of tissue from each time point were incubated with rabbit preimmune serum and then with anti-rabbit IgG-fluorescein conjugate as a control to judge the specificity of the immunochemical localization. No green fluorescence was seen in the cells of any of these sections (Fig. 7). Yellow and orange autofluorescence were observed and it followed the same pattern seen in sections treated with the peroxidase-specific antiserum. That is, phenolic material was seen in the walls and vacuoles of cells wound-healed for 3 d. Less of this autofluorescence was observed in tissue wound-healed for 4 and 5 d but longer periods of wound-healing resulted in increased autofluorescence. Figure 7 shows the periderm region from 6 d-wound-healed tissue treated with preimmune serum and anti-rabbit IgG-fluorescein conjugate. The region shown in Figure 7 is the same as that shown in Figure 8 which does show a green fluorescence. Thus the immunofluorescence showed the expected specificity and therefore the green fluorescence is a reliable indication for the localization of the anionic peroxidase.

Many cells in the periderm region had undergone division and were separated by new walls. A green fluorescent layer indicating the presence of anionic peroxidase was also seen on the inside of these new, relatively thin walls (Fig. 8). The green fluorescence was often visible only as one layer probably because the plane of the sectioning did not reveal clearly both sides across the middle lamella. Occassionally the sectioning plane clearly revealed immunofluorescence on both sides of the wall. For example, Figure 9 clearly shows that the fluorescent label was present on the inner sides of a wall common to two cells as well as on the inner wall of an adjoining cell.

Occasionally the outermost wall of a cell on the wound-healed surface was cut at a tangential angle revealing a broad region of the inner wall. Such a surface from potato tissue wound-healed for 5 d is shown in Figure 10 with a dramatic display of green fluorescence indicating the presence of anionic peroxidase which seems to be in close association with the microfibrils of the wall. Yellow autofluorescence due to phenolics is seen in the background in the primary portion of the wall.

The parenchyma cells of tissue wound-healed for 6 d showed no green fluorescence in the cell walls. However, the stromal sheath of starch grains showed green fluorescence. The sheath around the starch grains in the parenchyma cells from tissue wound-healed from 1 to 5 d also exhibited green fluorescence but no fluorescein was observed in the walls of these cells. The sheaths of starch grains in the freshly cut tissue had almost no fluorescence (as in Fig. 1).

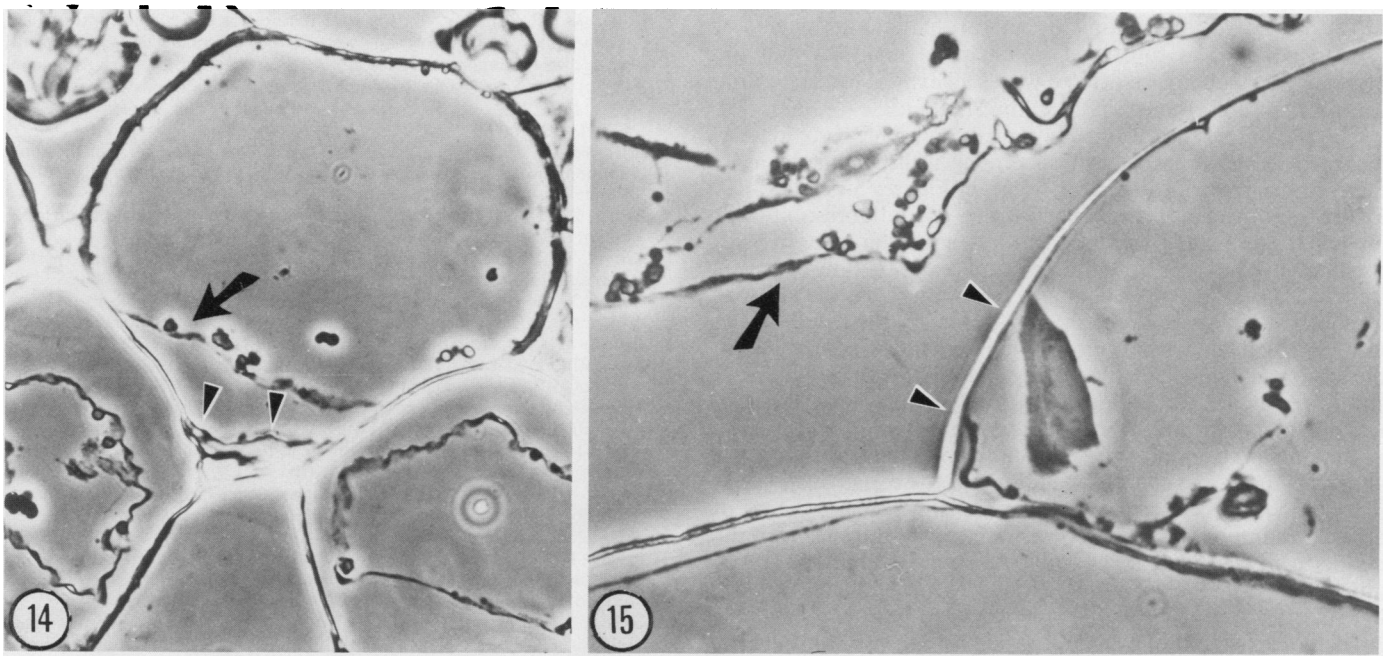
Phase contrast micrographs of the same sections examined by fluorescence microscopy were taken to exhibit details of the cellular structure. Figure 14 shows the same cells seen in Figure 6 and Figure 15 shows the same cells seen in Figures 7 and 8.

Staining of Wound-Healing Potato Tissue with Rabbit Anti-Isoperoxidase and Anti-Rabbit IgG-Rhodamine Conjugate. Previous reports have indicated that there can be some difficulty in distinguishing autofluorescence from specific immunofluorescence when utilizing fluorescein-coupled antibodies in plant tissue (15, 17). For this reason we examined the wound-healed potato tissue with fluorescence microscopy after staining the tissue with anionic peroxidase specific antibody and goat anti-rabbit IgG-rhodamine conjugate. Cell walls near the wound surface of freshly cut potato or tissue wound-healed for 1 d showed no fluorescence although the protein sheath around many of the starch grains did exhibit a slight reddish-orange fluorescence. One or more days after cutting, the outermost surface of the wound appeared brightly fluorescent. The fluorescence of the outer surface of the wound appears to be a result of some nonspecific interaction as it was also seen in control tissue treated with preimmune serum. Sections stained with rhodamine G alone gave a similar response when used on cut surface when the stain was applied a day or more after cutting. In all these cases the staining was confined to the outer surface and never reached other walls of even the outermost layer of cells.

Tissue which had been wound-healed 2 d or more exhibited weak fluorescence in the wall and cytoplasm/plasmalemma of almost all periderm cells. Although this fluorescence was not seen in tissue treated with preimmune serum, it is not clear that it was a peroxidase-specific fluorescence. An antibody-specific reaction was first seen in the periderm walls of tissue which had been wound-healed for 4 d and the bright orange-red fluorescent staining was observed in the same regions of cell wall as those found with fluorescein fluorescence seen in Figure 4. Similar bright fluorescent regions were seen in the periderm walls of tissue wound-healed for 5 d. Tissue which had been wound-healed for 6 d exhibited bright fluorescence in the walls of all periderm cells. The deposition of anionic peroxidase along the inside of the cell wall shown by the green fluorescent layer seen in Figure 6 was also revealed by the bright orange-red fluorescent layer observed in tissue wound-healed for 6 d. When the cut was made through the appropriate plane the inner surface on both sides of a wall between two cells showed orange-red fluorescence (Fig. 11) as observed with fluorescein in Figure 9. The double wall seen in Figure 11 is the same wall which exhibited green fluorescence with fluorescein-conjugate staining shown in Figure 8.

With the exception of the outermost wall there was almost no fluorescence visible in the control tissue treated with preimmune serum and as indicated above this fluorescence represents some nonspecific physical interaction between the wall and rhodamine. In parenchyma cells treated with the IgG-rhodamine conjugate there was bright orange-red antibody-specific fluorescence in the sheath of the starch grains, but no bright fluorescence in any of the parenchyma walls.

Autofluorescence and Acridine Orange Staining of Periderm Cells in Wound-Healing Potato. Thin sections of wound-healing tissue were examined in the fluorescence microscope without antibody treatment to monitor the development of autofluorescence. There was very little detectable autofluorescence until 3 d of wound-healing when fluorescent deposits were seen along the cell walls and in the vacuoles of the periderm. The intensity of this fluorescence was lower in tissues wound-healed for 4 or 5 d and dramatically higher intensity was observed in tissue which had been wound-healed for 6 d. Increased amounts of deposition of phenolic components in the cell walls of wound-healing tissue was also indicated by fluorescence microscopy of tissue stained with acridine orange. Cells in the periderm layer of tissue which had been wound-healed for 0 to 2 d had bright orange walls (Fig. 12) indicating that their walls were composed primarily of carbohydrates (5), while cell walls from tissue wound-healed for 6 d had bright yellow walls (Fig. 13). The walls of periderm cells



FIGS. 14 and 15. Phase contrast micrographs of potato tuber periderm cells from tissue which had been wound-healed for 6 d. Areas of cell wall that showed immunofluorescence are indicated by arrowheads. Cytoplasm pulled away from wall in many areas due to plasmolysis. Areas which exhibited autofluorescence are indicated by arrows. Figure 14 (×500) shows the same cells as Figure 6, and Figure 15 (×800) shows the same cells as Figures 8 and 11.

from tissue which had been wound-healed for 3 d were primarily orange but these walls did exhibit the first indications of yellow fluorescence in the walls—usually in the region of cell wall junctions. The periderm cell walls of tissue wound-healed for 4 and 5 d also showed predominantly orange fluorescence with some yellow and it was not until d 6 that these walls exhibited bright yellow fluorescence after acridine orange staining (Fig. 13).

Time Course of the Development of Peroxidase Activity in Wound-Healing Potato Tuber Slices. The periderm layer was manually removed from tissue slices which had been wound-healed for various periods from 0 through 6 d. Acetone powder was made from the isolated periderm tissue and the acetone powder samples were extracted with high salt. The extracts were dialyzed and assayed spectrophotometrically for peroxidase activity. Peroxidase activity increased 16-fold during a 6-d wound-healing period, with the largest increase seen between 4 and 6 d (Table I).

To measure the change in activity of the suberization-associated anionic peroxidase, the acetone powder extracts were analyzed with an immunobinding assay (16) utilizing the rabbit antibody specific for the anionic peroxidase. This assay revealed that there was a 14-fold increase in anionic peroxidase protein during the 6 d wound-healing period and there was the largest increase in peroxidase protein content between 4 and 6 d of wound-healing similar to the increase seen with the spectrophotometric assay (Table I).

Table I. *Peroxidase Activity of Acetone Powder Extracts of the Periderm Layer from Slices of Potato Wound-Healed for 0 through 6 Days*

Activity was determined by colorometric assay with guaiacol/H_2O_2 as substrate and enzyme protein level by immunobinding utilizing antibody specific to the suberization-associated anionic peroxidase and is reported as amount of activity per g or mg of acetone powder.

Days Wound-Healed	Anionic Isoperoxidase Level	
	Activity	Protein
	$\Delta A_{470}/min \cdot g$	$cpm\ ^{125}I/mg$
0	72	210
1	92	
2	372	190
3	612	670
4	595	700
5	646	1180
6	1175	2970

DISCUSSION

The results presented in this paper clearly demonstrate an increase in the suberization-associated anionic peroxidase during wound-healing of potato tuber slices. When the fluorescein conjugated antibody system was used green fluorescence indicating the presence of anionic peroxidase was first seen in the walls of periderm cells which had been wound-healed for 3 d (Fig. 3). This specific fluorescence increased in frequency and intensity from d 3 through d 6 (Figs. 3–6 and 8–10). A similar time course was observed when the goat anti-rabbit IgG-rhodamine conjugate was utilized. Specific orange-red fluorescence was first seen in the walls of periderm cells at d 4 and increased in frequency and intensity in tissue which was wound-healed for 5 and 6 d (Fig. 11). The time course of appearance of the immunochemically detected peroxidase was consistent with the changes in the peroxidase activity level measured in the periderm layer and with the changes in the anionic peroxidase protein level measured by the immunoblot assays. The present results agree in general with the earlier observations that an anionic peroxidase increased specifically in the periderm layer in a time course that correlated with the cytochemically detected suberization (4). A similar time course was observed for ABA induced synthesis of this anionic peroxidase in potato tissue culture (7). The synthesis of this anionic peroxidase also correlated with suberization in the tissue culture. All of the results thus far obtained show that the development of diffusion resistance of the wound surface of tuber

slices, the deposition of aliphatic and aromatic components of suberin polymer as measured by chemical analyses, and the production of suberin-associated waxes all exhibit the same time course as the appearance of the anionic peroxidase (6, 8, 21, 30).

The present results also show that the anionic peroxidase is localized in the inner side of suberizing walls. With the IgG-fluorescein staining this is clearly seen in Figures 5–6 and 8–10. Figure 9 shows adjacent cells each with a green fluorescent layer on the inside wall. Figure 11 exhibits a similar pattern with rhodamine fluorescence. Ultrastructural examination of suberized walls in potato tuber periderm had shown that the lamellar layers characteristic of suberin are located on the inside of the wall (9, 30). The fact that the anionic peroxidase is localized in the same region in which the suberin polymer is deposited argues strongly that the peroxidase is involved in the biosynthesis of this polymer and that this peroxidase is most probably involved in the polymerization of the phenolic components, to form the aromatic matrix of suberin. In addition to the earlier suggestions (22, 26) many recent reports have demonstrated the presence of aromatic components in suberin enriched preparations from the periderm of wound-healed potato (7), *Agave americana* crystal idioblasts (12), green cotton fiber (31), the endodermis and hypodermis of corn roots (24), the roots of bean (28), and the envelopes generated by tomato locule protoplasts (25). Furthermore, histochemical tests showed both lipid and phenolic components in hypodermal and endodermal cell walls of roots (23, 27, 29) which have been shown chemically and ultrastructurally to be suberized (20). That the formation of the aromatic polymer domains of suberin involves the anionic isoperoxidase was also suggested by a recent study on iron deficient bean plants. Such plants showed a severely depressed level of aliphatic and aromatic components characteristic of suberin. Attempts to elucidate the enzymic step blocked by iron deficiency revealed that the enzymes involved in the production of aliphatic components of suberin were not lacking but the anionic isoperoxidase levels were suppressed (28); upon supplementation of the growth medium with iron this enzyme level reverted to normal.

The change from orange to yellow fluorescence observed in the walls of wound-healing potato tissue stained with acridine orange (Figs. 12 and 13) may be due to the deposition of aromatic components into the suberin polymer. Cell walls which are primarily composed of cellulose have been shown to fluoresce orange when stained with acridine orange while the thick cell walls of bundle sheaths showed bright yellow fluorescence (5). Although this yellow fluorescence was attributed to the aromatic components of lignin (5), bundle sheath walls have been shown chemically to contain characteristic aliphatic components of suberin (10). The yellow fluorescence observed in the walls with acridine orange staining of the bundle sheath cells and the potato wound periderm may be due to the aromatic domains of suberin. Although there is no direct proof that aromatic components are covalently attached to the aliphatic components found in suberized walls, many pieces of indirect evidence summarized elsewhere (19, 20) strongly support the basic idea that suberin contains aromatic and aliphatic domains. The present results strongly support this notion and the previously proposed role for the anionic peroxidase in the synthesis of the aromatic domain of suberin.

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