Abscisic Acid Stimulation of Suberization[1]

INDUCTION OF ENZYMES AND DEPOSITION OF POLYMERIC COMPONENTS AND ASSOCIATED WAXES IN TISSUE CULTURES OF POTATO TUBER

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ABSTRACT

Effect of abscisic acid (ABA) on suberization of potato (*Solanum tuberosum* var. Russet-Burbank) tuber tissue culture was studied by measuring deposition of suberin components and the level of certain key enzymes postulated to be involved in suberization. ABA treatment resulted in a 3-fold increase in the polymeric aliphatic components of suberin and a 4-fold increase in the polymeric aromatic components. Hydrocarbons and fatty alcohols, two components characteristic of waxes associated with potato suberin, increased 9- and 5-fold, respectively, as a result of ABA treatment. Thus, the deposition of the polymeric aliphatics and aromatics as well as waxes, all of which have been postulated to be components of suberized cell walls, was markedly stimulated by ABA. ω-Hydroxy-fatty acid dehydrogenase which showed a rather high initial level of activity increased only 60% due to ABA treatment. Phenylalanine ammonia-lyase activity reached a maximum at a 5-fold level after 4 days in the ABA medium, whereas the control showed only a 3-fold increase. ABA treatment also resulted in a dramatic (7-fold) increase in an isozyme of peroxidase which has been specifically associated with suberization. Thus, ABA appears to induce certain key enzymes which are most probably involved in suberization.

ABA is generally regarded as a depressor or inhibitor of physiological and biochemical events in plants (24). In contrast to the large number of reports on inhibitory effects of ABA, there are few reports concerned with stimulatory effects. Recently, it was shown that ABA promoted cell division and DNA synthesis in Jerusalem artichoke tuber tissue culture (18).

We concluded that ABA stimulated suberization in potato tuber tissue based on the observation that ABA was released into the washing medium during the first day after cutting and that ABA at least partially reversed the inhibition of suberization caused by washing (22). In support of this conclusion, ABA was found to induce the deposition of polymeric aliphatic components characteristic of suberin in potato tissue culture. More recently, it was realized that suberin is probably composed of polymeric materials containing aromatic and aliphatic components and that waxes associated with suberin probably provide the water vapor diffusion barrier to suberized cell walls (23). If the previously observed ABA stimulation of deposition of polymeric aliphatic material in potato tissue culture represented induction of suberization, the synthesis of other components which are thought to be part of suberized cell walls should also be induced by ABA. In this paper we show that ABA in the medium of potato tuber tissue culture stimulates the deposition of aliphatic and aromatic polymeric materials and waxes, all of which are characteristic of suberized cell walls. Effects of ABA on the levels of certain enzymes thought to be involved in suberization are also reported.

MATERIALS AND METHODS

Tissue Cultures. Callus cultures were prepared from tuber parenchyma of *Solanum tuberosum* var. Russet-Burbank and maintained in modified Murashige-Skoog medium containing 2,4-D, 3 mg/l and kinetin, 0.03 mg/l (22). Calli were transferred to the same medium or medium containing 10^{-4} M ABA and at 2-d intervals after transfer, calli were removed and ground in −20°C acetone with a mortar and pestle. The slurry was filtered with Whatman No. 42 paper, the insoluble material was reground in cold acetone and filtered again. The acetone powder was air dried, stored at −20°C and used as the source of all enzyme preparations. The acetone extract was evaporated to a small volume under reduced pressure, transferred to a separatory funnel, acidified water was added, and extracted with $CHCl_3$ (3 × 50 ml).

Since deposition of suberin aliphatics had previously been found to be optimal at 10^{-4} M ABA (22), this concentration was used for all experiments, which were repeated three times with similar results. All data presented represent typical results and are from the same set of cultures.

Chemicals. Coenzymes, *p*-hydroxybenzaldehyde, vanillin, phenylalanine, and ABA (grade II) were from Sigma. Syringaldehyde and ω-hydroxypalmitic acid were from Aldrich. Other chemicals were of reagent grade. All solvents were of reagent grade and were redistilled prior to use except for acetone, diethyl ether and formic acid. 16-Hydroxy-[G-^{3}H]hexadecanoic acid (277.4 Ci/mol) was prepared and purified as previously described (2).

Enzyme Assays. Protein concentrations were measured by a dye binding method (5) using Coomassie blue and γ-globulin as standard, both supplied by Bio-Rad.

PAL[3] was assayed by a spectrophotometric method at 40°C (26). Acetone powder was stirred for 1 h at 4°C in 0.1 M borate buffer (pH 8.8) in the ratio of 10 mg/ml, and the mixture was centrifuged for 10 min at 10,000*g*. The supernatant (0.5 ml, containing 0.15–0.3 mg protein) was added to 2.5 ml of reaction mixture containing 100 μmol borate buffer (pH 8.8) and 30 μmol phenylalanine. Absorbance was monitored at 290 nm with readings taken at 20-min intervals. Rates were linear with respect to

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[3] Abbreviation: PAL, phenylalanine ammonia-lyase.

protein concentration up to at least 0.1 mg/ml and for up to 80 min of incubation.

ω-Hydroxy-fatty acid dehydrogenase activity was measured by a radiotracer assay (2). Acetone powder was stirred for 1 h at 4°C with 0.1 M Na-phosphate buffer (pH 6.7) containing 50 mM mercaptoethanol, in the ratio of 5 mg/ml. The mixture was centrifuged as above and 100 μl of the supernatant was added to a reaction mixture containing 0.1 mM 16-hydroxy-[G-^{3}H]hexadecanoic acid, 0.5 mM NAD, 0.5 mM NADP, 0.1 M glycine-NaOH buffer (pH 9.5), 1 mM dithioerythritol, and 1 mM $MgCl_2$ in a total volume of 1 ml. The mixture was incubated at 30°C for 30 min and the reaction was terminated by addition of 0.5 ml 6 N HCl. Unlabeled 16-hydroxypalmitate and hexadecane-1,16-dioic acid were added as internal standards. Total lipids were extracted with $CHCl_3$, the extract was dried under N_2, and redissolved in 0.3 ml tetrahydrofuran. Aliquots (10 μl) were assayed for ^{3}H and 50-μl aliquots were applied to 0.5-mm silica gel G plates and the chromatograms were developed in hexane:diethyl ether:formic acid (70:30:2). The lipids were visualized by spraying the chromatogram with 0.1% ethanolic dichlorofluoroscein, the silica gel from the region containing hexadecane-1,16-dioic acid was scraped and the radioactivity was measured by liquid scintillation spectrometry. Rates were linear with respect to protein concentration at least up to 90 μg/ml and for up to 60 min of incubation. The activity of ω-hydroxy-fatty acid dehydrogenase from acetone powder of freshly cut Russet-Burbank potato tuber was measured as above.

Activities of peroxidase isozymes were determined after separation by the previously described continuous 6% polyacrylamide gel electrophoretic system (4). Acetone powder was stirred with 0.04 M K-acetate buffer (pH 4.3) in the ratio of 20 mg/ml and the supernatant obtained as above was used as the enzyme source. Electrophoresis was performed with an LKB 2117 Multiphore apparatus. Gels were preelectrophoresed for 30 min. After application of protein samples (in a volume between 4–8 μl) current was maintained at 45 mamp for 9 h with continuous cooling by circulating cold (0°C) water. Gels were stained for peroxidase activity with 3-amino-9-ethylcarbazole and H_2O_2 (prepared according to Graham *et al.* [10] except that immediately before use, the staining solution was filtered with Whatman No. 42 paper and H_2O_2 was added after filtration) and fixed with H_2O:CH_3OH:acetic acid (50:50:7). Gels were scanned with a Helena Laboratories Quick Scan R and D instrument at 570 nm.

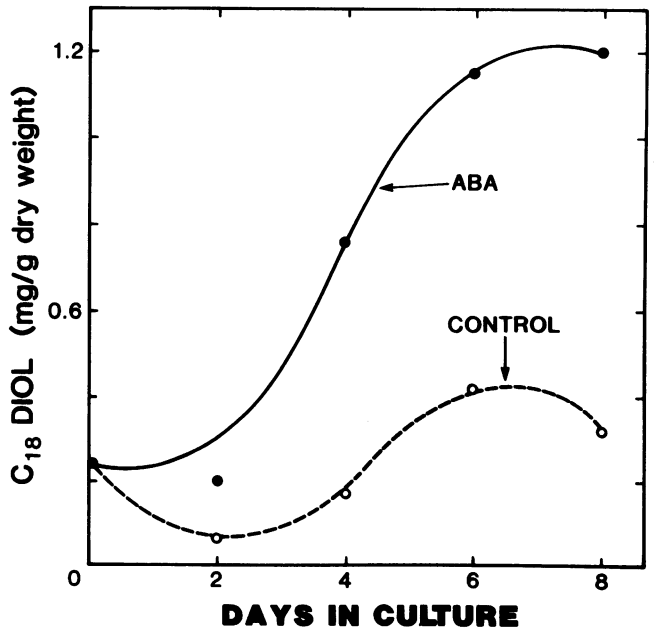


FIG. 1. Time-course of deposition of suberin aliphatics as measured by the amount of C_{18} diol released by $LiAlH_4$ treatment of the insoluble polymeric material from ABA-treated and control potato tissue cultures. The ABA concentration was 10^{-4} M and other experimental details are given in the text.

Analysis of Suberin and Associated Waxes. Acetone powder was Soxhlet extracted for 48 h with $CHCl_3$. Acetone powder, which had been extracted with buffer, as indicated above, was lyophilized and pooled with unextracted samples prior to Soxhlet extraction. The lipids recovered from acetone extracts (as above) were pooled with Soxhlet extracts and evaporated to dryness. The residue, representing total lipids from the tissue, was refluxed with 14% BF_3 in CH_3OH (w/w) for 6 h. The reaction mixture was transferred to a separatory funnel, H_2O added and the products were extracted with $CHCl_3$ (3 × 30 ml). The extract was dried, chromatographed on silica gel G with double development using hexane as the first solvent, followed by hexane:diethyl ether:formic acid (35:15:1). The hydrocarbon and fatty alcohol fractions were analyzed by combined GC/MS.

Portions of Soxhlet-extracted acetone powders were subjected to alkaline nitrobenzene oxidation (6). Reaction mixtures were acidified, extracted with diethyl ether, and chromatographed on silica gel G (6). The aromatic aldehyde fraction recovered from the silica gel was analyzed by HPLC with a μBondapak C_{18} column using H_2O:CH_3OH:acetic acid (93:5:2) containing 18 mM ammonium acetate. To measure polymeric aliphatics, the solvent extracted acetone powder, along with ω-hydroxypalmitate as an internal standard, was refluxed with $LiAlH_4$ in tetrahydrofuran for 48 h. The excess reagent was decomposed and the products were extracted and chromatographed on silica gel G as before (16). The diols were eluted from the gel with a 2:1 mixture of $CHCl_3$ and CH_3OH, converted to trimethylsilyl (TMS) ethers (16) and analyzed by GC/MS. The amount of octadec-9-ene-1,18-diol was used as a measure of polymeric aliphatics.

Gas Chromatography/Mass Spectrometry. A Hewlett-Packard 5840A gas chromatograph fitted with a coiled glass column (60 cm long × 2 mm i.d.) packed with 3% OV-101 on 100 to 120 mesh Ultrabond 20M was used. Column temperature was maintained at 200°C and injector temperature at 280°C. Helium was used as the carrier gas at a flow rate of 38.5 ml/min and the column effluent was directly introduced into a Hewlett-Packard 5985 mass spectrometer equipped with a computer data system. Silylated hexadecane-1,16-diol (generated from the internal standard) and octadec-9-ene-1,18-diol were identified by their mass spectra, which were recorded at 70 ev. Quantitation was based on peak areas of total ion chromatograms.

Radioactivity Measurement. Samples were assayed for ^{3}H in scintillation fluid containing Omnifluor (4 g/l, New England Nuclear), 30% ethanol and 70% toluene, using a Packard Tri-Carb 3255 Liquid Scintillation Spectrometer. The efficiency of ^{3}H counting was typically about 20%.

RESULTS

Deposition of Polymeric Aliphatics. In order to establish whether ABA stimulated suberization in tissue cultures, portions of thoroughly extracted acetone powders were subjected to exhaustive reduction with $LiAlH_4$ and the amount of octadec-9-ene-1,18-diol (C_{18} diol) liberated was used as a measure of polymeric aliphatics since this diol is known to be the major product derived from potato suberin (16). As shown in Figure 1, after a lag of 2 d, tissue cultures treated with ABA showed deposition of C_{18} diol-generating components (ω-hydroxyoleate and the corresponding dicarboxylic acid), reaching a plateau after 6 d at about 1.15 mg/g dry weight. Control cultures also showed deposition of some C_{18} diol-generating components after a lag of 4 d. After 6 d, control cultures reached a peak of deposition of C_{18} diol-generating

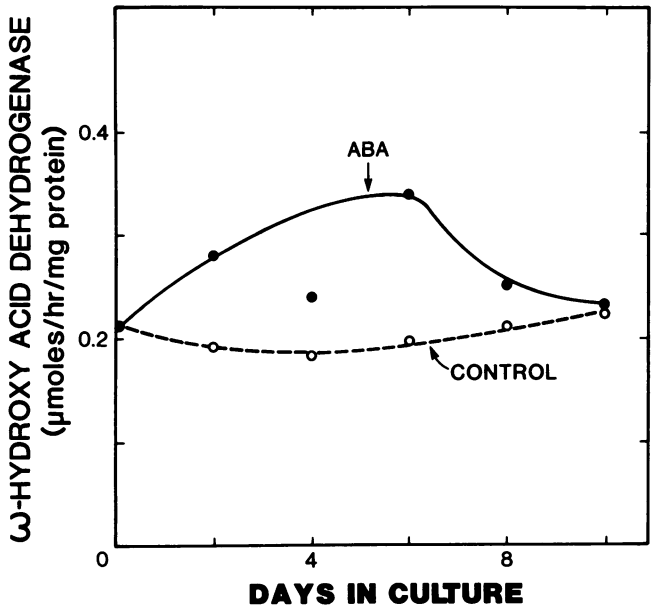


FIG. 2. Time-course of appearance of ω-hydroxy-fatty acid dehydrogenase activity in ABA-treated and control potato tissue cultures. The ABA concentration was 10^{-4} M and the enzyme activity in acetone powder extracts were measured as described in the text.

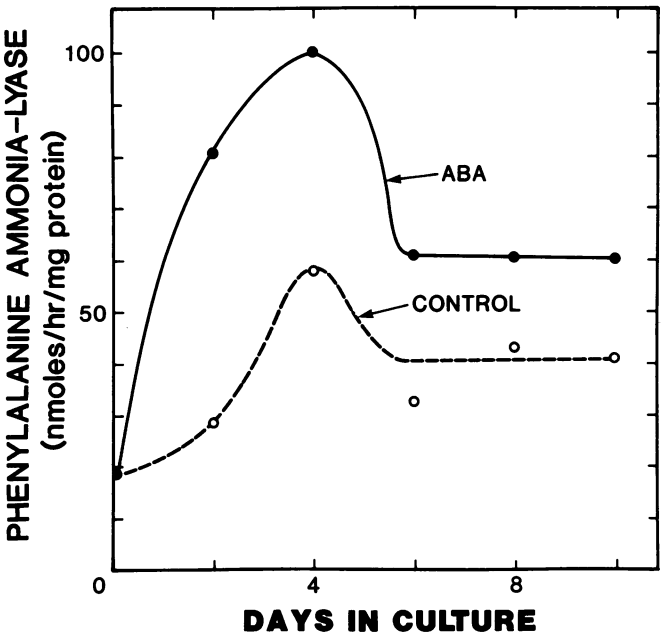


FIG. 3. Time-course of appearance of PAL activity in ABA-treated and control potato tissue cultures. The ABA concentration was 10^{-4} M and the enzyme activity in acetone powder extracts was measured as described in the text.

components at 0.42 mg/g dry weight, followed by a slight decline thereafter to 0.32 mg/g dry weight on day 8. In order to determine whether the age of the culture or ABA treatment affected the proportion of ω-hydroxyoleate and the corresponding dicarboxylic acid present in the polymeric material, the ratio of the two components was determined using deuterium labeling by substituting $LiAlD_4$ in place of $LiAlH_4$ (15). In all cases, about two-thirds of the C_{18} diol liberated was derived from the dicarboxylic acid.

ω-Hydroxy-Fatty Acid Dehydrogenase Activity. In contrast to the sharp stimulation of deposition of C_{18} diol-generating components by ABA, there was not a corresponding increase in activity of ω-hydroxy-fatty acid dehydrogenase, the enzyme which is thought to be involved in the synthesis of the dicarboxylic acid (Fig. 2). The initial level of activity of this enzyme was at about 0.2 μmol/h·mg protein and remained at approximately that level in controls throughout 10 d of culture. ABA-treated cultures showed a rise in activity to a level of about 0.34 μmol/h·mg protein on day 6, followed by a decline to control levels by the 10th d of culture. To determine whether the relatively small increase in the activity of this enzyme was due to a high initial level, fresh potato tuber tissue was examined for the activity level of the enzyme. The activity was barely detectable in this tissue whereas the initial level of activity in the tissue cultures was rather high as indicated above.

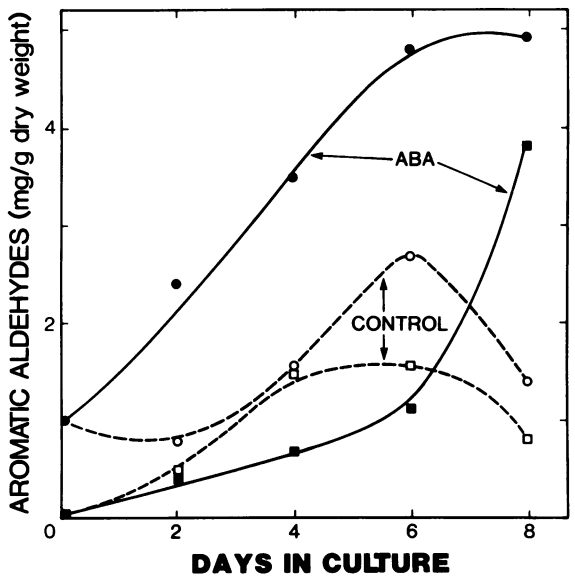
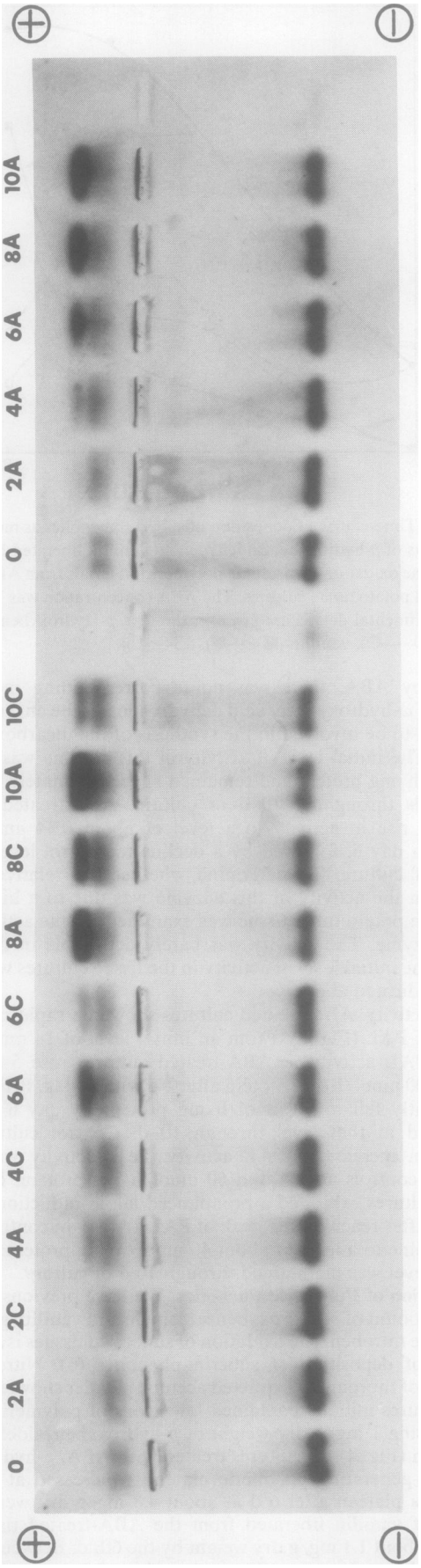


FIG. 4. Time-course of deposition of suberin phenolics as measured by the amounts of *p*-hydroxybenzaldehyde and vanillin liberated by alkaline nitrobenzene oxidation of insoluble polymeric materials from ABA-treated and control potato tissue cultures. The ABA concentration was 10^{-4} M and other experimental details are given in the text. *p*-Hydroxybenzaldehyde (●—●○--○); vanillin (■—■), (□--□).

PAL Activity. ABA-treated cultures showed a rapid rise in the activity of PAL (Fig. 3). From an initial level of 18 nmol/h·mg protein, PAL activity in ABA-treated cultures rose 5-fold to a peak of 100 nmol/h·mg protein after 4 d with no lag. PAL activity subsequently fell to 60 nmol/h·mg protein by day 6 and was maintained at that level through 10 d. Control cultures also showed an increase in PAL activity. Peak activity was 3-fold higher in controls after 4 d at 60 nmol/h·mg protein. However, control cultures exhibited a pronounced lag in induction of PAL activity. After reaching the peak of PAL activity in controls, there was a decline to a level of about 40 nmol/h·mg protein on day 6 and this level was maintained through 10 d of culture.

Deposition of Polymeric Phenolics. We have previously shown that the amount of *p*-hydroxybenzaldehyde and vanillin generated by alkaline nitrobenzene oxidation of suberized tissues is a reliable measure of deposition of suberin phenolics (6). Nitrobenzene oxidation of thoroughly extracted acetone powder showed that the tissue cultures initially contained low levels of polymeric phenolics, releasing 1 mg/g dry weight of *p*-hydroxybenzaldehyde and no vanillin (Fig. 4). In cultures treated with ABA, *p*-hydroxybenzaldehyde-generating polymeric materials increased at a steady rate with a plateau after 6 d at about 4.8 mg/g dry weight. The amount of vanillin liberated from the ABA-treated tissue rose slowly to about 1.1 mg/g dry weight by the 6th d. Between the 6th and 8th d, liberation of vanillin rose to about 3.7 mg/g dry weight.



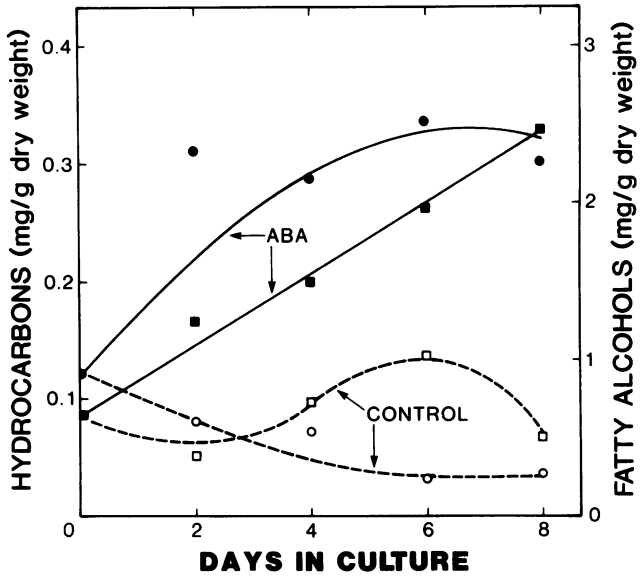


FIG. 6. Time-course of deposition of suberin-associated waxes in ABA-treated and control potato tissue cultures. The concentration of ABA was 10^{-4} M and other experimental details are given in the text. Hydrocarbons (●—●, ○- - -○); fatty alcohols (■—■, □- - -□).

Phenolic deposition in control cultures also increased. The amount of *p*-hydroxybenzaldehyde liberated rose to 2.7 mg/g dry weight by the 8th d, whereas liberation of vanillin rose to about 1.5 mg/g dry weight on the 6th day and declined to 0.5 mg/g dry weight by the 8th d. Overall, total deposition of suberin phenolics, as measured by liberation of *p*-hydroxybenzaldehyde and vanillin by nitrobenzene oxidation, was 3- to 4-fold greater in ABA-treated cultures than that in control cultures after 8 d.

Peroxidase Activity. Isoperoxidases from acetone powder extracts of tissue cultures were separated electrophoretically (Fig. 5). As each slot on the gel contained 2 μg total protein, relative specific activities can be compared directly from the intensity of the staining. Cathodic peroxidases tended to show a slight decline in activity with time of culture and there appeared to be no effect of ABA on the pattern of distribution of these isozymes. Three anodic peroxidases were observed, just as previously found by Borchert (3). Of these three peroxidases, the two slower migrating isozymes appeared to remain at essentially constant levels of activity in the control and ABA-treated cultures. The most rapidly migrating anodic band, corresponding to isozyme A4 of Borchert, was virtually absent initially, but began to appear after 2 d of culture and its activity increased about 5-fold by the 4th d in both control and ABA-treated cultures. By the 6th d, however, ABA-treated cultures showed an increase in the activity of A4 of about 30-fold over the initial level while controls showed no further increase. The activity of A4 in ABA-treated cultures nearly leveled off after 8 d and after 10 d its activity was about 70 times the initial level of activity. After 4 d of culture, control levels of A4 increased such that after 10 d its activity was about 10 times the initial activity. The right side of the gel in Figure 5 shows the activity of isoperoxidases from ABA-treated cultures only, where each slot again contained 2 μg total protein. Scanning of the gels showed that A4 increased in activity in sigmoidal fashion through

FIG. 5. Induction of an isoperoxidase by ABA treatment of potato tissue cultures. The left side of the gel shows comparison of isoperoxidase activities from ABA-treated and control cultures. The right side of the gel shows progressive changes in isoperoxidase activities from ABA-treated cultures only. Each slot on the gel contained 2 μg total protein and other experimental details are given in the text. In each case the number indicates the number of days in culture; C, control; A, ABA-treated.

10 d of culture in the presence of ABA.

Deposition of Suberin-Associated Waxes. Total deposition of hydrocarbons and fatty alcohols (representing both wax esters and free fatty alcohols) is shown in Figure 6. In ABA-treated cultures, fatty alcohol deposition increased approximately linearly from an initial value of 0.6 mg/g dry weight to 2.5 mg/g dry weight after 8 d. Control cultures accumulated fatty alcohols up through 6 d to about 1 mg/g dry weight followed by a decline to half that value by the 8th d. In both ABA-treated cultures and controls, the major fatty alcohols were *n*-alkanols of even chain length from C_{20} to C_{30} with C_{22} being the most abundant component.

Cultures treated with ABA accumulated hydrocarbons through 6 d, followed by a plateau. In the ABA-treated cultures, hydrocarbon content increased from an initial level of 0.12 mg/g dry weight to about 0.26 mg/g dry weight. Controls showed a steady decline in hydrocarbon content and after 8 d of culture the level reached was about 0.03 mg/g dry weight. In all cases, *n*-alkanes with odd numbers of carbon atoms (C_{19}-C_{29}) were the major components.

DISCUSSION

The present finding of ABA stimulation of deposition of polymeric aliphatic material is in agreement with the results of Soliday *et al.* (22). ABA stimulated aliphatic deposition about 3-fold over the control level, whereas control cultures appeared to suberize to a small extent. Similarly, phenolics and waxes tended to be deposited in control cultures. We attribute the slight stimulation of suberization in control cultures to injury of cells caused by the callus transfer process.

Phenolic deposition in tissue cultures treated with ABA, as measured by generation of *p*-hydroxybenzaldehyde and vanillin by alkaline nitrobenzene oxidation was about 4 times that of controls after 8 d of culture. As before (6), in no case was syringaldehyde detected as a major product of nitrobenzene oxidation of tissues, indicating that lignification was not the process being observed.

If the ABA-induced synthesis of polymeric components characteristic of suberin represents true suberization, it would be expected that wax synthesis would be induced by ABA as well. Inasmuch as hydrocarbons and fatty alcohols are the most unique constituents of the wax in potato suberin (23), we used the accumulation of these two as a measure of wax deposition. The amounts of fatty alcohols and hydrocarbons found in ABA-treated tissues were 5- and 9-fold greater, respectively, than the amounts found in controls after 8 d of culture. The ratio of total hydrocarbons to total fatty alcohols was 0.12, a value within the range found for periderm waxes from a variety of subterranean storage organs (9). Also, chain lengths of hydrocarbons and fatty alcohols in ABA-treated cultures were the same as those found in periderm waxes (9). Thus, the hydrocarbons and fatty alcohols from ABA-treated cultures most probably represented suberin associated waxes.

The presence of dicarboxylic acids is a rather unique feature of suberin and the two enzymes responsible for oxidizing ω-hydroxy-fatty acids are ω-hydroxy-fatty acid dehydrogenase and ω-oxo-fatty acid dehydrogenase. Of these, only ω-hydroxy-fatty acid dehydrogenase was found to be induced by wounding in potato tuber tissue (1). Inasmuch as ABA in the medium of potato tissue cultures induced deposition of C_{18} diol-generating components of which about two-thirds were dicarboxylic acids, it might be expected that ABA would induce development of ω-hydroxy-fatty acid dehydrogenase activity. However, ABA treatment caused only a relatively small increase in the activity of this enzyme in the tissue cultures. The lack of a dramatic increase in the level of this enzyme appears to be explained by the presence of a rather high initial level of activity of this enzyme in the tissue culture when compared to fresh Russet-Burbank tuber tissue in which this enzyme activity was barely detectable. Under the optimum conditions of the *in vitro* assay used, the total catalytic potential of ω-hydroxy-fatty acid dehydrogenase in 6-d control tissue, for example, was over 3,200 times as much as that required to synthesize the amount of dicarboxylic acid actually deposited. Even if *in situ*, only a small fraction of the potential activity of ω-hydroxy-fatty acid dehydrogenase was capable of being expressed, that level of activity would have been more than adequate to account for all of the C_{18} dicarboxylic acids actually produced. The reason for the high initial activity of ω-hydroxy-fatty acid dehydrogenase activity in the potato tissue cultures is unknown. In any case, in the present tissue culture system, unlike that in wound healing tuber slices, ω-hydroxy-fatty acid dehydrogenase does not appear to be regulating the synthesis of the dicarboxylic acids of suberin.

The influence of ABA on PAL induction has been studied in tissue cultures of *Helianthus tuberosus* (19) and *Phaseolus vulgaris* (12), seeds of *Lactuca sativa* (7) and embryonic axes of *Phaseolus vulgaris* (25). In the tissue culture systems ABA had no promotive effect on PAL activity, whereas in lettuce seeds, ABA strongly inhibited (greater than 80%) the appearance of PAL. In *P. vulgaris* axes, ABA stimulated peak PAL activity very slightly (17%). Inasmuch as PAL is a key enzyme in phenylpropanoid metabolism (13) and phenylpropanoids are known to be present in suberin (20), ABA stimulation of suberization might also involve induction of PAL activity. We found peak stimulation of PAL activity in potato tissue culture to be 1.7 times the control level after 4 d of culture and ABA sustained higher PAL activity than in controls through 10 d. The pattern of PAL activity induced by ABA is very similar to the pattern of PAL activity induced by wounding alone (21). Indeed, the pattern of PAL activity in control cultures tends to support the possibility that transferring of tissue cultures mimics wounding of fresh tissue.

The influence of ABA on isoperoxidase activities has been studied in several systems. In tobacco tissue culture and germinating mung bean cotyledons, ABA severely inhibited peroxidase activity (8, 17), whereas in Jerusalem artichoke tissue culture, ABA slightly stimulated peroxidase activity (19). In lignifying *P. vulgaris*, ABA had no effect on peroxidases (12). Inasmuch as it is generally agreed that cross-linking of phenolic monomers in lignifying tissues is mediated by peroxidases (11) and as suberin appears to contain up to 40% phenolics (14), by analogy to lignification, peroxidases may be involved in suberization. In fact, an anodic species of peroxidase was found to be induced in wound-healing potato tissue and this isozyme (A4) was found specifically in the cell layers undergoing suberization (3). Our results clearly show that in the present tissue culture an anodic peroxidase (A4) was induced by ABA. The time-course of appearance of A4 in ABA-treated cultures follows the time-course of phenolic deposition. Although it cannot be stated with certainty that A4 is involved in suberization, the magnitude of its induction by ABA, its time-course of appearance and high degree of correlation with the time-courses of deposition of polymeric phenolics and aliphatics, suggest that it plays a role.

The results presented in this paper constitute the first instance where deposition of all three postulated components of suberized cell walls has been examined. The finding that ABA induces the deposition of polymeric aliphatic and aromatic components as well as that of associated waxes supports the tentative model proposed for the structure of suberized cell walls and confirms the hypothesis that ABA does in fact induce suberization of cell walls in potato tissue. The observed induction of enzymes thought to be involved in suberization by ABA provides an example where this hormone stimulates a physiological response. The induction of isoperoxidase A4 by ABA appears to be a clear example where this hormone is probably inducing the synthesis or processing of

an enzyme. Therefore, this system might be suitable for elucidating a mechanism of action of ABA at the molecular level.

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