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Abscisic Acid Localization and Metabolism in Barley Aleurone Layers

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ABSTRACT

Aleurone layers of *Hordeum vulgare*, cv. 'Himalaya' took up [^{14}C]-abscisic acid (ABA) when incubated for various times. Radioactivity accumulated with time in a low speed, DNA-containing pellet accounting for 1.6 to 2.3% of the radioactivity recovered in subcellular fractions at 18 hours. Thin layer chromatography of ethanolic or methanolic extracts of the cytosol, which contained greater than 95% of the radioactivity taken up by layers, revealed that labeled ABA was metabolized to phaseic acid (PA) and 4'-dihydrophaseic acid (DPA) and three polar metabolites Mx_1, Mx_2, and Mx_3. ABA was not metabolized by endosperm, incubated under conditions used for layers, indicating that metabolism was tissue-specific. Layers metabolized [^{3}H]DPA to Mx_1 and Mx_2. ABA, PA, and DPA-methyl ester and epi-DPA-methyl ester inhibited synthesis of α-amylase by layers incubated for either 37 or 48 hours. These layers converted the methyl DPA and epi-methyl-DPA esters to their respective acids. DPA did not inhibit *Lactuca sativa* germination or root and coleoptile elongation of germinating *Hordeum vulgare* seeds, or coleoptile elongation of germinating *Zea mays* seeds.

GA_3 promotes the synthesis and release of hydrolases by barley aleurone layers and ABA counteracts the effects of GA_3 (21). Although the effects of ABA on synthesis of hydrolases in barley aleurone layers have been well described, there have been no published reports of experiments dealing with uptake, localization, and metabolism of ABA by this tissue. This report describes such experiments and, in addition, compares the effects of ABA with one of its metabolites, DPA, on the development of α-amylase activity in aleurone layers as well as on germination of seeds and growth of seedlings. A preliminary report on certain aspects of the present work has appeared (3).

MATERIALS AND METHODS

Preparation of Aleurone Layers. *Hordeum vulgare*, cv. 'Himalaya,' seeds (1972 and 1974 crop, Washington State University, Pullman) were deembryonated and converted to half-seeds (1). Half-seeds were treated with 10 to 20% Clorox for 15 min and then rinsed with 500 ml sterile water. Half-seeds were incubated on sterile sand or on a double layer of Whatman No. 1 filter paper in Petri dishes wrapped with aluminum foil for 3 to 4 days at 22 C. Aleurone layers were removed from the half-seeds and incubated as described in subsequent sections.

ABA Metabolism. Twenty aleurone layers were incubated in 1.4 ml of solution containing 10 mM $CaCl_2$, 2 mM Na-acetate buffer (pH 5.0), 70 μg chloramphenicol, 1.38×10^5 dpm *S*-[^{14}C]-ABA or *R*-[^{14}C]ABA and ±1 μM GA_3. The solutions were contained in 25-ml Erlenmeyer flasks which were shaken in the dark at 26 C in a Dubnoff metabolic incubator at setting 6. Starchy endosperm from 15 aleurone layers were treated in the same way except that 1.8×10^5 dpm *RS*-[^{14}C]ABA was used in the incubation. The layers were rinsed several times with incubation medium and aliquots of the medium plus rinsings were taken to determine total ABA uptake. The layers or endosperm were homogenized in 90% methanol with a mortar and pestle. The homogenate was filtered and the residue reground with 90% methanol and then extracted with 90% ethanol at 60 C for 1 min. The combined filtrates were taken to dryness *in vacuo* at 35 C. The residue was taken up in a small volume of methanol and spotted onto 0.25-mm-thick Merck precoated Silica Gel F-254 TLC plates. The plates were developed in benzene-butanol-acetic acid (70:25:5) or chloroform-methanol-acetic acid-water (40:15:3:2). Radioactivity was located by autoradiography; the silica gel was scraped from the plate and eluted with 90% ethanol. Radioactivity was estimated by scintillation counting using a 10-ml solution of toluene-Triton X-100 (2:1) containing 4 g/l Omnifluor (New England Nuclear).

Subcellular Localization. Thirty aleurone layers were incubated in 3 ml of solution containing 10 mM $CaCl_2$, 2 mM Na-acetate buffer (pH 5.0), 150 μg chloramphenicol, 1.1×10^6 dpm *RS*-[^{3}H]ABA (22 Ci/mmol), 5 μM *RS*-ABA, and ±1 μM GA_3. The layers were shaken in 25-ml Erlenmeyer flasks as previously described for varying periods of time. After incubation, layers were rinsed with cold incubation medium lacking ABA. The medium and washes were combined and aliquots taken to determine uptake.

The rinsed layers were chopped with a razor blade and then ground with a mortar and pestle in 2 ml of solution containing (pH 7.4) 50 mM Tris-HCl, 0.4 M sucrose, 1 mM $MgCl_2$, 10 mM KCl, and 1 mM DTT. The homogenate was filtered through Miracloth and the residue reground in 1 ml of the same solution. The combined filtrates were centrifuged at 750 or 1,000*g* for 10 min, and the supernatants at 12,000*g* for 20 min. The resultant supernatants were either centrifuged at 38,000*g* for 90 min or 200,000*g* for 40 min. The pellets from each of the centrifugations were resuspended in 3 ml homogenization medium and repelleted under the original conditions. The pellets from the second centrifugation were resuspended in 1 ml 1% Triton X-100 and counted. Aliquots of the 38,000 or 200,000*g* supernatants were also counted and the remainder acidified to pH 2.5 and extracted with 1-butanol. The butanol-extracted material was chromatographed on TLC plates as described under ABA metabolism.

Assay of α-Amylase. Ten layers were incubated in 2 ml containing 20 mM Na-succinate buffer (pH 5.0), 100 mM $CaCl_2$, 100 μg chloramphenicol, 20 nM GA_3, and various concentrations of RS-

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Table I. Distribution of Radioactivity in Cell Fraction

Thirty layers incubated with shaking in 3 ml of solution containing 10 mM $CaCl_2$, pH 5.0 2mM Na-acetate buffer, 150 μg chloramphenicol, 1.1 X 10^6 dpm [^{3}H]-(RS)-ABA (22 Ci/mmol), 5 μM (RS)-ABA and ± 1 μM GA_3; following incubation layers rinsed with above solution lacking ABA; layers chopped with a razor blade and ground with a mortar and pestle in 2 ml solution containing pH 7.4 50 mM Tris-HCl, 0.4 M sucrose, 1 mM $MgCl_2$, 10 mM KCl and 1 mM DTT; homogenate filtered through Miracloth and the residue re-ground in 1 ml of the same solution; combined filtrates centrifuged at 750 or 1,000 g 10 min and supernatants at 12,000 g 20 min; 12,000 g supernatants centrifuged at 200,000 g 40 min; pellets from each centrifugation re-suspended in 3 ml homogenization medium and re-pelleted; final pellets re-suspended in 1 ml 1% Triton X-100 and counted; aliquots of 200,000 g supernatants also counted; distribution of radioactivity also determined at 4, 5, 11 and 15 hr; representative data of three experiments shown.

Fraction	Addendum	Percent Radioactivity Recovered		
		6 hr	12 hr	18 hr
1,000g pellet				
	-GA_3	1.3	1.9	2.3
	+GA_3	0.6	1.2	1.6
12,000g pellet				
	-GA_3	0.3	0.3	0.4
	+GA_3	0.2	0.2	0.2
200,000g pellet				
	-GA_3	0.9	0.5	0.7
	+GA_3	0.4	0.5	0.8
200,000g supernatant				
	-GA_3	97.5	97.3	96.6
	+GA_3	98.8	98.1	97.4

ABA, PA,[4] DPA, DPA, DPA-methyl ester, or epi-DPA-methyl ester. Layers were incubated at 26 C in the dark for 37 or 48 h. The α-amylase activity was determined in both layers and incubation medium by the methods of Chrispeels and Varner (1, 2).

Germination and Growth Studies. *Zea mays* (hybrid yellow, W. Atlee Co., Warmingster, PA.) seeds were germinated for 90 to 96 h in the dark except for a 15-min red light treatment each day (11). After removal of the apical 3-mm, 1-cm coleoptile sections were excised and the leaf rolls removed. Ten coleoptile sections were incubated in 5-cm Petri dishes in 4 ml either 5 mM (pH 6.0) Na-succinate buffer or 100 mM (pH 6.8) citric acid-200 mM Na-phosphate (5), 10 mM IAA, and various concentrations of either ABA or DPA. Incubation was in the dark for 24 h at 22 C.

Seventy mg of lettuce seeds (*Lactuca sativa* L., cv. Grand Rapids, J. Harris Co., Rochester, N. Y.) were sown in 5-cm Petri dishes on two sheets of Whatman No. 1 filter paper moistened with 2 ml (pH 7.0) 1 mM citric acid-2 mM Na_2HPO_4 and varying concentrations of GA_3, ABA, and DPA. Incubation was in the dark at 22 C for 41 or 48 h.

Barley seeds were sown in batches of 15 on two layers of Whatman No. 1 filter paper in 125-ml Erlenmeyer flasks containing 2 ml H_2O to which were added varying concentrations of Ga_3, ABA, or DPA. Seeds were germinated in the dark at 22 C for 24 and 48 h at which time per cent germination, coleoptile, and root lengths were determined.

DNA Assay. DNA was quantitated wtih diphenylamine and acetaldehyde according to Giles and Myers (6) with calf thymus DNA (Schwarz/Mann, Orangeburg, N.Y.) serving as a standard. Since aleurone layers contain pentosans composed of xylose and arabinose (12), D(+)-xylose and L(+)-arabinose were also assayed with diphenylamine, to insure that they did not interfere.

Chemicals. *RS*-[^{3}H]ABA (25) and [2-^{14}C]ABA (19) were synthesized. [^{3}H]DPA, [^{3}H]DPA-methyl ester, and [^{3}H]epi-DPA-methyl ester were obtained by reducing PA (7). [^{14}C]PA was obtained from bean embryos (20).

[4] Abbreviations: DPA: 4'-dihydrophaseic acid; PA: phaseic acid.

RESULTS

ABA Localization in Layers. The distribution of radioactivity in subcellular fractions obtained by differential centrifugation from layers incubated with [^{3}H]ABA is presented in Table I. A low level (2.3%) of radioactivity accumulated with time in the 750 or 1,000*g* pellets. This radioactivity could not be removed by resuspension and repelleting of the 750 or 1,000*g* pellets which contained a diphenylamine-positive substance(s), presumed DNA. The 12,000, 38,000, and 200,000*g* pellets each contained less than 1% radioactivity at all times examined and radioactivity did not accumulate within them. Greater than 95% of the radioactivity recovered in cell fractions was found in the 200,000*g* supernatant.

ABA Metabolism in Layers. Initial experiments revealed that a large proportion of radioactivity was not partitioned into 1-butanol from aqueous solutions at pH 2.5. Consequently, in time course experiments utilizing either *S*-[^{14}C]ABA or *R*-[^{14}C]ABA, we spotted filtered ethanolic or methanolic homogenates directly onto TLC plates. Each sample was developed in two different solvent systems as described under "Materials and Methods" in order to separate and quantify six radioactive bands. Aleurone layers incubated in *S*-[^{14}C]ABA removed 88% of the radioactivity from the medium by 8 h while layers incubated in *R*-[^{14}C]ABA took up 61% by 8 h and 82% by 24 h. Figure 1 presents the time-dependent changes in [^{14}C]ABA and its metabolites for layers fed the two enantiomers. The *S*-[^{14}C]ABA was rapidly metabolized with only 3 to 4% remaining in the tissue after 8 h (Fig. 1B). Besides PA and DPA there were three other metabolites observed which we designate as Mx_1, Mx_2, and Mx_3. Mx_1 and Mx_2 are major metabolites constituting 57% and 25%, respectively, of the radioactivity recovered at 24 h. The time courses of their formation suggest the possibility that MX_1 was derived from Mx_2. The *R*-[^{14}C]ABA appeared to be metabolized in a manner qualitatively similar to *S*-[^{14}C]ABA but at a slower rate. We also incubated isolated endosperm with *RS*-[^{14}C]ABA. The uptake was quite poor (10%) compared with aleurone layers and of the ABA taken up over 24 h, only 10% was metabolized.

Since the time course of ABA metabolism by the aleurone

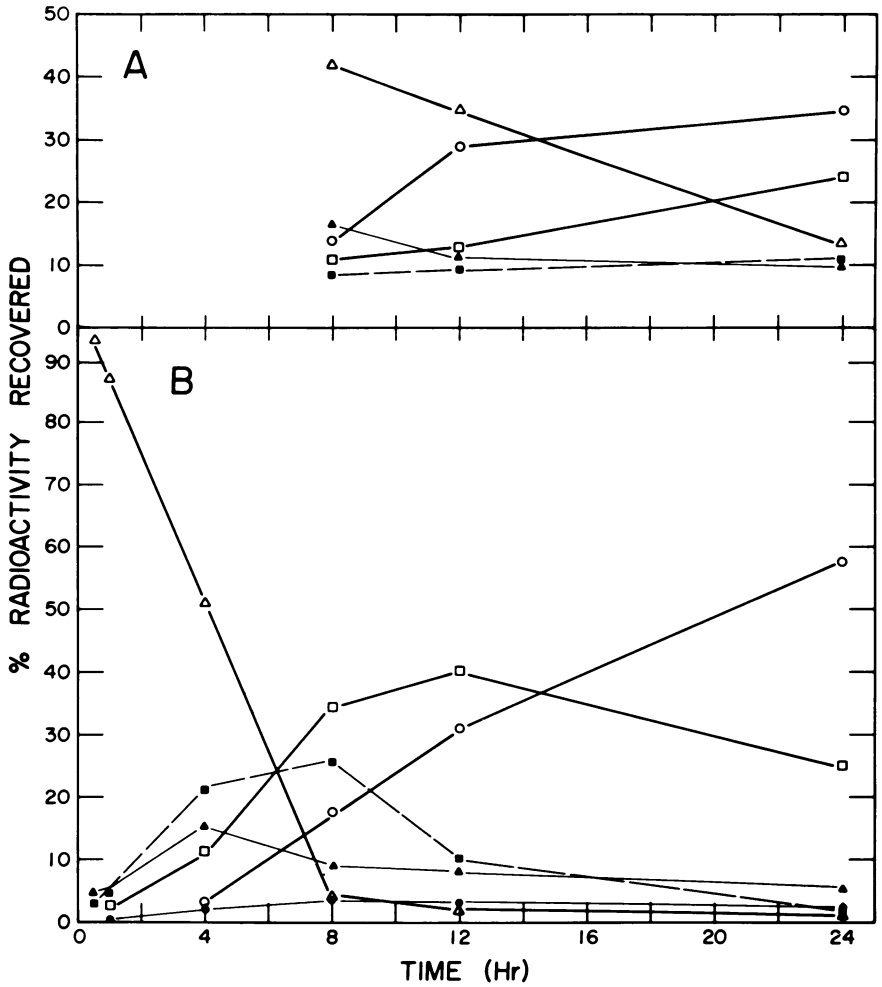

FIG. 1. A, B: Time-dependent metabolism of *R*-[^{14}C]ABA (A) and *S*-[^{14}C]ABA (B) by aleurone layers. Procedures for layer incubation and homogenization, TLC of ethanolic or methanolic homogenates and determination of radioactivity in metabolites summarized under "Materials and Methods"; (△——△): ABA; (■——■): PA; (▲——▲): DPA; (○——○): Mx_1; (□——□): Mx_2; (●——●): Mx_3.

layers indicated that the metabolites Mx_1 and Mx_2 might be derivatives of DPA, we incubated layers with [^{3}H]DPA. Figure 2 shows that the DPA was completely converted to two compounds with chromatographic mobilities similar to those of Mx_1 and Mx_2.

Biological Activity of ABA Metabolites. Tables II and III summarize the effects of ABA, PA, DPA-methyl ester, and epi-DPA-methyl ester on GA_3-stimulated α-amylase activity in the medium at 37 and 48 h. Activity was enhanced 6.2-fold at 0.02 μM GA_3 by 37 h (Table II). This enhancement was completely abolished by 0.05 μM ABA and was inhibited 70 and 82% at 0.26 and 0.60 μM DPA-methyl ester. Incubation of layers with a combination of 0.02 μM GA_3 and 0.2 or 0.56 μM epi-DPA-methyl ester suppressed activity 67 and 57%, respectively.

By 48 h 0.02 μM GA_3 promoted α-amylase activity 8.6-fold. Activity was inhibited 82 to 85% from 0.2 to 2 μM ABA. While PA suppressed activity by 86% at 0.2 μM, it completely abolished activity at 2 μM. Whereas α-amylase activity was not detected in the medium following incubation with 1.2 μM-DPA-methyl ester, activity was inhibited 43% at 0.17 μM ester.

Tissue extract of layers which took up 19% DPA-methyl ester during a 41-h incubation in 0.02 μM GA_3 plus 0.64 μM ester contained 64% less α-amylase than extract from layers incubated in GA_3 alone.

To determine whether DPA- and epi-DPA-methyl esters were metabolized in those layers assayed at 37 h for enzyme release, layers were ground in methanol, the homogenates spotted onto TLC plates and chromatographed in chloroform-methanol-acetic acid-water (40:15:3:2). Five zones of radioactivity were found following feeding with either DPA- or epi-DPA-methyl esters. Nine and 10% of the radioactivity was recovered as DPA and epi-DPA, respectively, with 63% (DPA) and 15% (epi-DPA) occurring as further metabolites (Mx compunds) of DPA and epi-DPA.

Elongation of barley coleoptiles was inhibited 40 and 100% at 0.1 and 100 μM ABA, respectively (Table IV, Exp. 1). At these ABA concentrations root elongation was inhibited 58 and 91%, DPA at 0.65 μM did not inhibit root elongation. In another experiment (Exp. 2), coleoptile elongation was inhibited 27 to 72% at 0.1 to 100 μM ABA. Whereas 0.22 μM DPA impaired coleoptile elongation 10%, 0.65 μM DPA was ineffective. Neither concentration of DPA inhibited root elongation. In a third experiment, 20 μM DPA did not prevent either coleptile or root elongation while 20 μM ABA suppressed these 35 and 69%, respectively.

Eight lettuce seed germination experiments with numerous concentrations of GA_3 by itself or together with ABA or DPA were carried out. Maximum stimulation of germination by GA_3 was at 100 μM, with complete reversal occurring at 10 μM ABA. Ten μM DPA was without effect.

The IAA at 10 μM promoted corn coleoptile elongation 1.18-fold (up to 1.24-fold in other experiments) and 60 μM ABA reversed this stimulation. DPA at 6, 20, and 60 μM DPA did not significantly affect elongation.

DISCUSSION

Do Aleurone Layers Metabolize ABA? Table I shows that 95% or more of the radioactivity recovered in subcellular fractions,

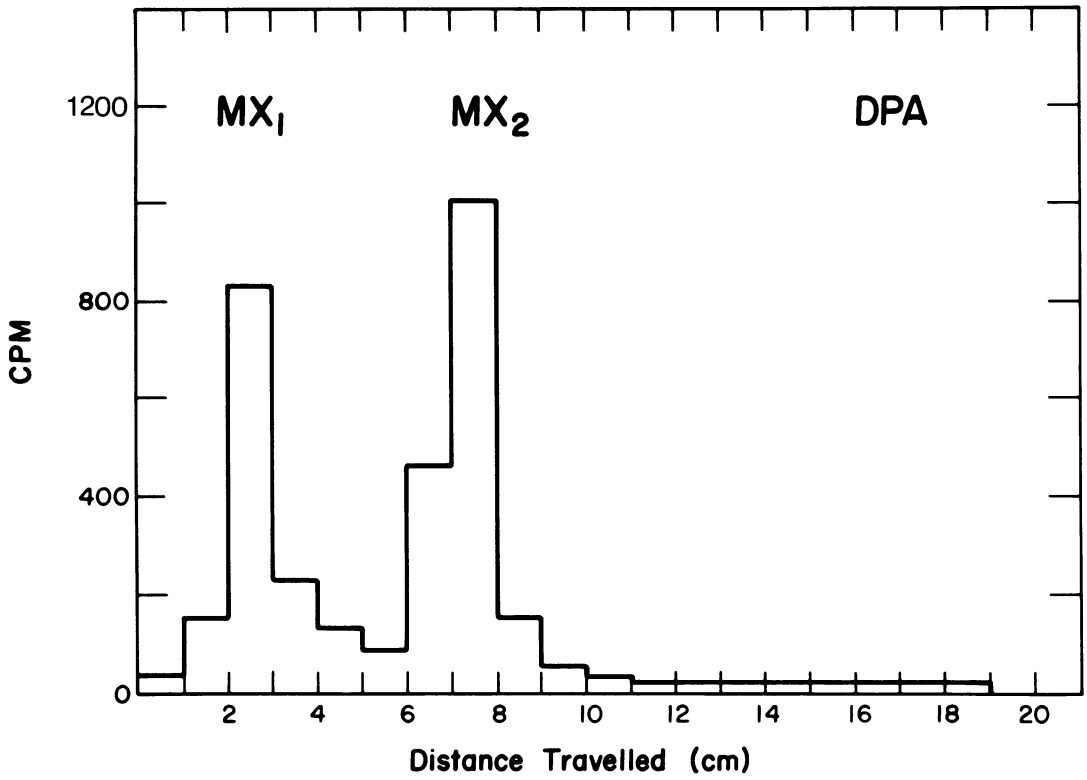

FIG. 2. Metabolism of [^{3}H]DPA by aleurone layers. Fifteen layers were incubated in 6.0 ml $CaCl_2$-acetate buffer-chloramphenicol as under "Materials and Methods" and 1,036,364 (24 h) or 955,181 (48 h) dpm [^{3}H]DPA; following incubation layers were washed three times with 2 ml incubation medium each time; layers were ground 5 min in 1 ml 90% aqueous acetone; homogenate was pulled through Miracloth; 0.1 ml of filtrate was counted; recoveries were 87.8% (24 h) and 95.5% (48 h); acetone was removed by evaporation under N_2; residue was taken up in 3 ml H_2O and extracted with chloroform-butanol as under "Materials and Methods"; aqueous phase was placed on TLC and developed in chloroform-methanol-acetic acid-water (45:15:3:2) after it was absorbed onto charcoal and desorbed with ammoniacal ethanol solution.

Table II. Effect of ABA, DPA and epi-DPA-methyl Esters on Release of α-amylase into Medium by 37 Hr Incubated Layers

Ten layers incubated in the dark at 26 C in 2 ml containing 20mM pH 5.0 Na-succinate buffer, 100 mM $CaCl_2$, 100 µg chloramphenicol and (RS)-ABA plus [^{3}H]-(RS)-ABA, [^{3}H] -DPA-methyl ester or [^{3}H] -epi-DPA-methyl ester; following incubation layers washed once with 1 ml and then 2 ml incubation medium; for uptake determinations, aliquots of the combined medium and washes added to a solution of toluene:Triton X-100 (2:1) containing 4 g/l Omnifluor and radioactivity estimated by liquid scintillation counting; α-amylase determined according to Chrispeels and Varner (1,2); one unit of enzyme is defined as a change of 1 absorbance unit/min at 620 nm; data are representative of two experiments, replicate experiment was carried out at 41 hr with only ABA and DPA-methyl ester.

Treatment	Uptake pmoles/layer	Units α-amylase
Minus GA_3		5.3
0.02µM GA_3		33.0
plus 0.05µM ABA	4.9	3.9
plus 0.26µM DPA-methyl ester	8.8	10.0
plus 0.60µM DPA-methyl ester	15.6	6.3
plus 0.20µM epi-DPA-methyl ester		10.8
plus 0.56µM epi-DPA-methyl ester		14.5

which were prepared for analysis of ABA localization, was present in the cytosol. This observation prompted an investigation of the time-dependent metabolism of *S*-[^{14}C]ABA by layers. The time course of labeling with *S*-[^{14}C]ABA (Fig. 1B) demonstrated a decrease in labeled ABA concomitant with appearance at 0.5 h of labeled PA and Mx_3 followed by labeled DPA and Mx_2 at 1.0 h. Significant radioactivity in Mx_1 was observed at 4 h. The turnover of PA suggests conversion to another compound with DPA or Mx_2 being the most likely product. The apparent origin of Mx_2 is DPA since incubation of layers with [^{3}H]DPA followed by TLC of tissue extracts yielded labeled Mx_1 and Mx_2 (Fig. 2). Because labeled Mx_1 appeared subsequent to the other ABA metabolites, it seems likely that Mx_1 is derived from Mx_2. The results from kinetic analysis taken together with those from [^{3}H]DPA feeding are consistent with the following catabolic pathway: ABA → PA → DPA → Mx_2 → Mx_1. A number of lines of evidence indicate

Table III. Effect of ABA, PA and DPA-methyl Ester on Release of α-amylase by Layers Incubated 48 hr

See legend to Table II for procedures utilized for layer incubation, uptake of ABA or its metabolites and α-amylase determination; representative data of three experiments shown.

Treatment	Uptake pmoles/layer	Units α-amylase
Minus GA_3		2.7
0.02μM GA_3		23.1
plus 0.20μM ABA	22	4.3
plus 2.00μM ABA	265	3.5
plus 0.20μM PA		3.3
plus 2.00μM PA		2.7
plus 0.17μM DPA-methyl ester	3.1	13.2
plus 1.20μM DPA-methyl ester	7.4	2.3

Table IV. Effect of ABA and DPA on Barley Seedling Coleoptile and Root Elongation

Batches of 15 barley seeds sown on two layers of Whatman No. 1 filter paper in 125 ml Erlenmeyer flasks containing 2 ml H_2O and the below concentrations of (RS)-ABA or DPA; seeds germinated in the dark at 22 C 48 hr; data are means of triplicate replications for each treatment, statistics = σ.

Addenda Concentration	Coleoptile Length (cm) Exp. 1	Exp. 2	Exp. 3
100 μM ABA	0.00	0.26 ± 0.13	
10 μM ABA		0.40 ± 0.05	
20 μM ABA			0.38 ± 0.13
0.10 μM ABA	0.50 ± 0.00	0.68 ± 0.31	
0.22 μM DPA		0.83 ± 0.37	
0.65 μM DPA		0.98 ± 0.37	
20 μM DPA			0.69 ± 0.08
None	0.84 ± 0.38	0.93 ± 0.30	0.58 ± 0.16
		Root Length (cm)	
100 μM ABA	0.17 ± 0.13	0.25 ± 0.07	
10 μM ABA		0.57 ± 0.49	
20 μM ABA			0.31 ± 0.01
1 μM ABA		1.20 ± 0.70	
0.10 μM ABA	0.66 ± 0.44	1.63 ± 0.83	
0.22 μM DPA		1.63 ± 1.33	
0.65 μM DPA	1.49 ± 0.67		
20 μM DPA			1.06 ± 0.16
None	1.55 ± 0.75	1.53 ± 0.66	0.99 ± 0.09

that Mx_3 is a direct derivative of ABA (Singh and Walton, unpublished). Conversion of ABA to DPA via PA has already been demonstrated to occur in embryonic bean axes (20, 24), water-stressed bean leaves, roots of *Phaseolus vulgaris* (23), and cell-free preparations of *Echinocystis lobata* liquid endosperm (7).

The presence of the three polar metabolites Mx_1, Mx_2, and Mx_3, extend the previous observation by Sondheimer *et al.* (18) that a polar metabolite, M_3, occurs in dormant and stratified *Fraxinum americana* embryos fed *S*-[2-^{14}C]ABA. This polar metabolite apparently arose from DPA and constituted 40% of the radioactivity recovered in ABA and its metabolites at 24 and 48 h. A compound with chromatographic properties similar to ash M_3 is also present in wilted and unwilted *P. vulgaris* L., cv. 'Red Kidney' beans constituting 14% of the radioactivity in ABA and its metabolites after 30-h incubation (10). The purification of the polar metabolites is the subject of a forthcoming paper (Singh and Walton, unpublished).

The results of the feeding experiments with *R*-[^{14}C]ABA (Fig. 1A) demonstrate that *R*-ABA can be metabolized by the same pathway as *S*-ABA, but at a slower rate (7). We have tentatively concluded this previously (17) but the possibility that the apparent *R*-ABA metabolism was due to contaminating *S*-ABA could not be excluded. In these experiments 70% of the total [^{14}C]ABA fed was metabolized. Since there is less than 5% *S*-ABA in the *R*-ABA (17), it is clear that the bulk of the ABA metabolized was the *R* enantiomer.

The metabolism of labeled ABA appears to be tissue-specific since endosperm incubated under conditions identical to those used for layers did not metabolize ABA. Since the presence of GA_3 is required to promote the synthesis and release of layer hydrolases, the possibility existed that ABA metabolism as well as localization and uptake differed for layers incubated with GA. While small quantitative differences (*e.g.* Table I) were noted for layers incubated with and without GA_3, the patterns observed for metabolism, localization, and uptake were similar.

Are PA and DPA Inactive Products of ABA Metabolism? The metabolism of ABA and PA is thought to inactivate hormone activity (15, 22). Although Kriedemann *et al.* (13) reported that PA inhibited photosynthesis, this inhibition appears not to be due to PA but to a contaminant (16). In the present work, PA obtained from bean embryos (20) inhibited activity of α-amylase from layers incubated 48 h (Table III). PA appears to be at least as effective as ABA (compare 2 μM PA and ABA, Table III). Ho (personal communication) has shown that PA inhibits ^{35}S incorporation into certain barley aleurone layer polypeptides separated by SDS-polyacrylamide gel electrophoresis. PA appears to be more active than ABA in this system. While abscission in the excised nodes of cotton seedlings is accelerated by PA, its activity is only 1/10 that of ABA (4). In addition, PA possessed only about 0.5% of the ABA growth inhibitory activity in the wheat embryo germination assay (14).

DPA-methyl ester also inhibited α-amylase activity in layers

incubated either 37 or 48 h (Tables II and III). Although DPA-methyl ester appeared to be more effective than ABA at 48 h (compare 1.2 μM DPA-methyl ester with 2.0 μM ABA in Table III), the ester was less so at 37 h. Since DPA-methyl ester was converted to DPA we do not know which of these species was responsible for the inhibition.

Walton and Sondheimer (24) noted that DPA inhibited increase in fresh weight of excised bean axes by only 6%. DPA did not impair lettuce seed germination or elongation of barley roots and coleoptiles (Table IV) and corn coleoptiles. Whereas DPA does not appear to be a general inhibitor of germination and elongation, it may inhibit α-amylase synthesis. This suggests that DPA may preferentially inhibit some enzyme synthesis without impairing growth or its related processes. Assay of other enzymes, *e.g.* endomannase (8, 9), in systems where DPA does not impair germination or elongation are required to support this suggestion. In as much as we have not crystallized either PA or DPA, the possibility that we are dealing with the effects of contaminants cannot be excluded.

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