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THE EFFECT OF LIGHT ON LIPOXYGENASE ACTIVITY IN DWARF PEA SEEDLINGS

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Abstract—Continuous illumination of 10-day-old etiolated dwarf pea seedlings caused an increase in lipoxygenase activity At the same time the activity in both stem and leaf tissue decreased The lipoxygenase isoenzymes of the whole seedling and separated leaf and stem tissue were affected differently by light It is concluded that lipoxygenase is not involved directly in photosynthesis or chloroplast development

INTRODUCTION

THE ENZYME, lipoxygenase (E.C.1.13 1.13) catalyses the peroxidation of compounds containing a methylene interrupted *cis,cis*-pentadiene structure. It is found in high concentrations in seeds[1] and other storage organs[2], and for some time was thought to be limited to these sources Recently, however, lipoxygenase activity has been detected in a range of leaves,[3–5] stem tissue[5] and *Chlorella pyrenoidosa*.[6] The ultimate function of lipoxygenase is still unknown, but the appearance of two lipoxygenase isoenzymes specifically in leaves of dwarf pea seedlings[5] suggests that it might have a role to play in photosynthesis or chloroplast development. The following experiments were undertaken to investigate this possibility further.

RESULTS

Pea seedlings that had been germinated and grown in the dark for 10 days were divided into three groups. One group was analysed and chromatographed immediately; the second was grown in the dark for a further 5 days before analysis; the third was illuminated and analysed after 1·5 and 5 days

Effect of light on total lipoxygenase activity

Between days 10 and 15 in the dark the lipoxygenase activity of whole pea seedlings declined. In the light there was a gradual progressive increase in lipoxygenase activity on a unit fresh weight, unit weight acetone powder and seedling basis. The specific activity declined, however, especially between 1·5 and 5 days illumination, (Table 1).

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TABLE 1. EFFECT OF CONTINUOUS LIGHT ON THE LIPOXYGENASE ACTIVITY IN ILLUMINATED AND DARK GROWN PEA SEEDLINGS

	Samples			
	D LO	L1 5	L5	D5
Lipoxygenase activity				
U g fr wt	378	446	586	132
U mg protein	129	121	84	65
U g acetone powder	8850	9880	10620	3540
U seedling	305	371	481	175
Chlorophyll content				
mg g fr wt	0	0 06	0 38	0
mg seedling	0	0 05	0 31	0

Sample D LO was obtained from seedlings that had been grown in the dark for 10 days Samples L1 5 and L5 from seedlings subsequently illuminated for 1 5 and 5 days respectively, sample D5 from seedlings grown in the dark for a further 5 days One unit, U, of lipoxygenase activity represents an uptake of 10 nmol O_2 min

Effect of light on the isoenzyme pattern

The lipoxygenase activity of each sample was separated into five active fractions on a CM cellulose column For each sample most of the protein was eluted in fraction A by the starting buffer. Generally the protein content of the various fractions was in decreasing order. A > E > B > C > D (Table 2) The percentages of total activity recovered from the column in each fraction are shown in Table 3. After 1 5 days in the light there was a decrease in activity in fraction A and increases in fractions B and E After five days however, the activity in fraction A had been recovered and only fraction C showed an increase over that found in the 10-day-old dark grown seedlings

Lipoxygenase activity and isoenzyme pattern of light-grown leaves and stems

After 6 days in the light, seedlings were excised as usual and their leaves and stems separated Leaf and stem tissue were then analysed separately in the usual way The activity in leaves was much higher than in stem tissue on a unit weight basis but much lower on a protein weight basis The two tissues contributed equally to the total activity of the seedling (Table 4). The activity of the stem tissue was almost all localized in fraction B (Table 4) This was reflected by the increased proportion of protein eluted in this fraction (Table 2) As before,[5] no activity was found in fractions C and D from stem tissue In leaves most

TABLE 2. PROPORTION OF TOTAL PROTEIN RECOVERED ELUTED IN EACH FRACTION FROM CM-CELLULOSE

Sample	Fraction protein (% recovered)				
	A	B	C	D	E
D LO	76	7	5	1	11
L1 5	54	13	11	3	19
L5	84	4	5	1	7
D5	62	10	10	1	18
LS	51	21	6	2	20
LI	89	3	3	0	5

Pea seedlings were grown in the dark for 10 days (sample D/LO) and subsequently kept in the dark for a further 5 days (D5) or illuminated for 1 5 days (L1 5), 5 days (L5) or 6 days and divided into stem (LS) and leaf (LL) tissue Fractions A–E were eluted respectively with 0 005 M acetate pH 5 7, 0 05 M acetate pH 5 7, 0 10 M acetate pH 5 7 0 15 M acetate pH 5 7, 0 1 M Na_2HPO_4

of the activity was divided between fractions A and C. There was activity in fraction D and also this time in fraction B, possibly due to contamination of the leaf samples with petiole tissue (Table 4).

TABLE 3 PERCENTAGE OF TOTAL LIPOXYGENASE ACTIVITY IN EACH FRACTION ELUTED FROM CM-CELLULOSE COLUMN FOR SAMPLES ILLUMINATED FOR VARIOUS TIMES

Sample	Fraction lipoxygenase activity (% recovered) A	B	C	D	E
D/LO	26	40	16	6	12
L1 5	9	49	15	8	19
L5	29	35	22	4	10
D5	9	44	19	5	24

For explanation of samples and fractions, see Table 2

DISCUSSION

Previous reports on the effect of light on the lipoxygenase activity of seedlings have suggested an inhibitory effect.[7] In light that keeps the far-red form of phytochrome above 1·25% of total phytochrome in mustard cotyledons, further synthesis of lipoxygenase is halted.[8] Oelze-Karrow *et al.* have used this to propose a threshold level control by phytochrome,[8] although since the discovery of hydroperoxide isomerase[9] all lipoxygenase assays involving absorption measurements at 234 nm have become suspect.

In pea seedlings, 5 days illumination increases the lipoxygenase content on a unit fresh weight and seedling basis by over 50%. At the same time, however, light is inhibiting stem growth and promoting leaf growth. Etiolated[5] and non-etiolated leaves have a much higher lipoxygenase activity on a unit fresh weight basis than does stem tissue. Therefore the increase in lipoxygenase activity in the light is probably entirely due to the increasing contribution to total activity by the leaf tissue. Indeed comparing Table 4 with ref. 5, light

TABLE 4 TOTAL LIPOXYGENASE ACTIVITY FROM ILLUMINATED LEAF AND STEM TISSUE AND ITS DISTRIBUTION ON CM-CELLULOSE

	Tissue (lipoxygenase activity) Leaf	Stem
U/g fr wt	619	164
U/mg protein	26	59
U/g acetone powder	5630	3490
U/seedling	117	103
Fraction	% Activity recovered	
A	38	5
B	8	84
C	36	0
D	4	0
E	14	12

Leaf and stem tissue was separated from seedlings illuminated for 6 days
Fractions A–E are explained in the legend to Table 2

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has reduced the activity of both leaf and stem tissue on a unit fresh weight basis In both tissues light induces a considerable reduction in activity of all fractions except one. In leaves fraction C is unaffected, and neither is fraction B in stem tissue

It seems from the results reported here that the presence of lipoxygenase in pea seedlings and particularly in leaves is not directly related either to photosynthetic processes or to chloroplast development Recently it has been found that lipoxygenase activity is associated with microbodies in oat and wheat leaves, and in potato tubers and other plant tissues[10] and it seems likely if lipoxygenase reflects microbody activity, this activity is not under the same control as chloroplast development It is interesting to note that lipoxygenase is also not under the same light-mediated control as peroxidase[11] an enzyme that can also catalyse the hydroperoxidation of fatty acids *in vitro* [1]

On illumination dwarf pea seedlings produce a stem-growth inhibitor, xanthoxin,[12 13] which has been shown to be formed *in vitro* from certain xanthophylls [14] Also from *in vitro* studies, Firn and Friend[15] suggested that lipoxygenase may be involved in the conversion of violaxanthin to xanthoxin Conditions favouring xanthoxin production do stimulate total lipoxygenase activity more than carotene-bleaching activity [11] It was suggested that one isoenzyme may be particularly involved in xanthoxin production[11] and the differential effect of light on the isoenzyme fractions described here may implicate fraction C in leaves in this conversion.

EXPERIMENTAL

Treatment of plant material Dwarf pea (*Pisum sativum*, var Meteor) seeds were planted in moist vermiculite and allowed to germinate in total darkness for ten days at 21 At this stage some seedlings were illuminated under a fluorescent desk lamp, illumination 215 lx (12 hr), 645 lx (12 hr) per day

Analysis of plant material At various times (as described in the Results) seedlings were excised above the cotyledons, weighed and acetone powders prepared from them In the case of green tissue the chlorophyll content was determined from the acetone supernatant [16] The powders were solubilized and partially purified as described previously [5] The partially purified samples were applied to CM cellulose columns and eluted sequentially with 0·005 M acetate pH 5·7 (fraction A), 0·05 M acetate pH 5·7 (fraction B), 0·10 M acetate pH 5·7 (fraction C), 0·15 M acetate pH 5·7 (fraction D), and 0·1 M Na_2HPO_4 (fraction E) [5] exactly as described before [17]

Protein was determined by the method of Lowry *et al* [18] and lipoxygenase was assayed polarographically as described previously [2 17] One unit (U) of activity indicates an uptake of 10 μmoles O_2 per min

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