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INHIBITION OF THE LIGHT-INDUCED VITAMIN K_1 AND PIGMENT SYNTHESIS BY ABSCISIC ACID

H. K. LICHTENTHALER and K. BECKER

Botanisches Institut der Universität Münster, Münster, Westfalen, Germany

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Abstract—Upon illumination of etiolated barley seedlings, vitamin K_1 is synthesized at the same time as the chlorophylls, thylakoids and carotenoids. The natural plant hormone abscisic acid depresses the light-induced formation of vitamin K_1, chlorophylls and carotenoids. The low isoprenoid synthesis in the dark (K_1, carotenoids) is not inhibited by abscisic acid. There is no specific inhibition in the synthesis of a single isoprenoid lipid. Thus the functional concentrations of the pigments and K_1 in chloroplasts are formed, even though the total amount synthesized is below normal. It is suggested that abscisic acid interferes with the induction of thylakoid formation which then results in a reduced isoprenoid synthesis.

INTRODUCTION

THE NATURAL plant hormone abscisic acid appears to be a ubiquitous regulator of plant growth. It has been detected in many different tissues, such as buds, leaves, seeds, fruits and tubers.[1-5] Abscisic acid exhibits multiple physiological effects; e.g. inhibition of seed germination and of flower initiation, induction of dormancy, acceleration of petiole abscission and of leaf senescence.[5]

The mode of action of abscisic acid is not known as yet. It seems to inhibit synthesis of the gibberellins[5,6] and depresses the formation of isoprenoid plastid lipids (carotenoids, chlorophylls and lipophilic benzoquinones).[7] In addition to benzoquinones, plastids contain the naphthoquinone vitamin K_1, which is associated in thylakoids with the pigment system I of photosynthesis.[8,9] The first of the quinones synthesized upon illumination of etiolated barley seedlings is vitamin K_1, whose synthesis thus preceeds that of the prenylated benzoquinones.[10]

In this paper, we describe the effect of abscisic acid on light-stimulated vitamin K_1 and pigment synthesis. Since chlorophylls, carotenoids and vitamin K_1 are all present in chloroplasts in certain relative concentrations which are important in relation to photosynthesis, the effect of abscisic acid on these functional lipid concentrations was investigated.

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RESULTS AND DISCUSSION

In a previous paper, it has been shown that illumination of etiolated seedlings particularly stimulates the synthesis of those isoprenoid lipids which are present in the dark only in low concentration. These are β-carotene, violaxanthin, neoxanthin and vitamin K_1. Their synthesis in light proceeds parallel to the formation of chlorophyll a and thylakoids.[10,11] Thus the lipid concentrations of etioplasts are changed to the functional lipid relations of photosynthetically active chloroplasts within 24 hr. The effect of abscisic acid on the light-induced isoprenoid synthesis was determined in a known system. Barley seedlings grown in complete darkness contain lutein as the main carotenoid component. Phylloquinone (vitamin K_1) is present in minor amounts (Table 1, column a). When the excised shoots are

TABLE 1. SYNTHESIS OF ISOPRENOID LIPIDS IN ETIOLATED BARLEY SHOOTS IN THE DARK

Lipid	Amount of lipid (μg in 50 shoots)*			
	(a)		(b)	(c)
Vitamin K_1	1·2	1·5	1·8	1·7
Carotenoids	18·8	22·4	30·9	31·8
β-Carotene	2·9	2·1	4·5	4·0
Lutein	11·5	14·5	18·0	19·0
Violaxanthin	3·0	3·9	6·0	5·5
Zeaxanthin	0·9	1·2	1·5	2·0
Neoxanthin	0·5	0·7	0·9	1·3

* (a) 6-day-old shoots, values from two separate determinations; (b) excised 6-day-old shoots dipped with the ends for 18 hr in 20% aq. methanol; (c) excised 6-day-old shoots dipped with the ends for 18hr in abscisic acid solution (10 ppm) in 20% aq. methanol.

kept in the dark in aqueous solution, the synthesis of isoprenoid lipids continues. After 18 hr darkness the level of vitamin K_1 is increased by 20 to 30% and the level of total carotenoids by 50–60% (Table 1, column b). The increase in the shoots treated with abscisic acid is of the same order (Table 1, column c) as in the control plants. Small differences found in the individual lipids are within the variability of the plant material used. The results from Table 1 clearly show that the isoprenoid synthesis in the dark is not inhibited by abscisic acid.

In contrast to this, the light-induced synthesis of vitamin K_1, chlorophylls and carotenoids is strongly depressed by abscisic acid. After 12 hr of illumination the synthesis of vitamin K_1, chlorophylls and carotenoids is only about half that of the untreated controls (Table 2). The ratio chlorophyll a–b and chlorophyll a–K_1 and the ratio chlorophylls–carotenoids are about the same in control and test plants (Table 2, 12 hr values). There is also good agreement in the percentage composition of the individual carotenoid components (Table 3, 12 hr values).

These observations clearly indicate that, despite the depression of total isoprenoid synthesis, there is no specific inhibition of any individual isoprenoid lipid. In fact, the way of lipid synthesis in abscisic acid treated plants does not differ from that of the control plants except in a quantitative manner. In the first 12 hr of illumination we find high chlorophyll a/b ratios. This seems to be due to the formation of mainly pigment system I of photosynthesis, which exhibits a high a/b ratio.[8,9,12] The preferred synthesis in the first hours of

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TABLE 2. EFFECT OF ABSCISIC ACID ON THE LIGHT-INDUCED SYNTHESIS OF CHLOROPHYLLS, VITAMIN K_1 AND CAROTENOIDS IN 6-day-old ETIOLATED BARLEY SEEDLINGS

Compound	Amount (μg in 50 shoots)			
	Control (etiolated plants + light)		Test (+abscisic acid) (etiolated plants + light)	
	12 hr	18 hr	12 hr	18 hr
Chlorophyll a	487·0	450·0	236·0	220·0
Chlorophyll b	102·0	150·0	50·0	68·0
Chlorophyll a + b	589·0	600·0	286·0	288·0
Vitamin K_1	6·5	9·0	3·5	5·0
β-Carotene	28·0	38·0	13·0	20·0
Lutein	65·0	80·0	40·0	39·0
Violaxanthin	15·0	28·0	10·0	13·3
Zeaxanthin	3·8	5·0	3·3	4·0
Neoxanthin	1·5	6·3	1·5	3·8
Carotenoids	113·3	157·3	67·8	80·3
Ratios				
a/b	4·8	3·0	4·7	3·2
a/K_1	76·0	50·0	67·5	44·0
$\frac{\text{Chlorophylls}}{\text{Carotenoids}}$	5·2	3·8	4·2	3·6

TABLE 3. THE EFFECT OF ABSCISIC ACID ON RELATIVE AMOUNTS OF CAROTENOIDS IN BARLEY SEEDLINGS

Compound	Carotenoids (% of total carotenoids by wt.)			
	Control (etiolated plants + light)		Test (etiolated plants + light)	
	12 hr	18 hr	12 hr	18 hr
β-Carotene	25·0	24·0	19·0	25·0
Lutein	57·0	51·0	59·0	49·0
Violaxanthin	13·2	17·8	14·9	16·3
Zeaxanthin	3·4	3·2	4·9	5·0
Neoxanthin	1·4	4·0	2·2	4·7
Carotenoids	100·0	100·0	100·0	100·0

illumination of vitamin K_1, β-carotene and violaxanthin, which are mainly associated with pigment system I, is in agreement with this view. During the further illumination period (12–18 hr) the total chlorophyll content of the excised barley shoots does not increase in controls or abscisic acid treated plants. The capacity to form further amounts of chlorophyll a is lost in excised barley shoots. In both cases there is, however, an augmentation in the chlorophyll b level, which is compensated by a decrease in the chlorophyll a content. Since chlorophyll a is the precursor of chlorophyll b[13] this is to be expected. The chlorophyll a/b ratios now change from 4·7–4·8 to 3·0–3·2 (Table 2). The decreasing a/b ratio indicate that now also pigment system II of photosynthesis is formed.

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In this second illumination period (12–18 hr) the vitamin K_1 level increases 1·4-fold in controls and in tested plants. K_1 contains, like chlorophyll, the phytyl side-chain. Thus the latter is still synthesized, though there is no new chlorophyll synthesis. The formation of carotenoids continues in this second light period, too. The total carotenoid content increases 1·2–1·4-fold. Among the individual compounds we find a particularly strong increase in the level of neoxanthin. This is further indication for the formation of pigment system II, since neoxanthin is preferably associated with this pigment system.[8,9] After 18 hr the relative concentrations of the individual lipids are approximately the same in control and test plants (Tables 2 and 3).

With respect to the lipid changes in the second illumination period there is, again, only a quantitative difference between controls and abscisic acid treated plants. The general tendency to build up a fully functional photochemical system is the same in both cases. The results reported here thus clearly indicate that abscisic acid does not inhibit the normal development of functional photosynthetic units. It reduces, however, the number of photosynthetic units that are formed. The light-induced chlorophyll and lipoquinone synthesis in plastids is directly correlated with protein synthesis and the formation of thylakoids.[10,11,14] Chlorophylls, carotenoids and, in general, also vitamin K_1, are quantitatively localized within the photochemical-active thylakoids.[8,9] The synthesis of these functional chloroplast lipids proceeds only to that degree as new thylakoids are formed. Since abscisic acid depresses pigment and quinone synthesis, it is likely, that it interferes with the formation and multiplication of thylakoids by inhibition of protein-synthesis.

It is generally accepted that light induces a gene activation which then gives rise to chlorophyll and thylakoid synthesis. The formation of functional chloroplasts from thylakoid-free etioplasts is, in fact, blocked by inhibitors of protein and RNA synthesis.[15] It is suggested here that abscisic acid depresses the light-induced gene activation. It is of interest in this respect that abscisic acid can act as inhibitor of protein and RNA synthesis.[5] Thus its mode of action seems to be similar to that of other natural plant hormones which are thought to be effectors or repressors of gene activation.

EXPERIMENTAL

Cultivation of Seedlings

Barley seedlings (*Hordeum vulgare* L.) were grown in water culture in the absolute dark at 20°. After 6 days the shoots, which showed an average length of 12 cm, were excised. Every ten shoots were dipped with the cut ends in a beaker (25 ml), which contained 10 ml of an (±) abscisic acid solution (10 ppm) in 20% aq. MeOH. The controls were placed in 10 ml 20% aq. MeOH. The whole procedure was carried out in a very dim, green, safety light (0·5 lx) using two Schott filters (B618 and GG14, thickness 4 mm). After an incubation time of 7 hr in the complete dark, the beakers were placed in the light (Osram-Fluora lamps 40 W, 2500 lx) for 12 and 18 hr.

Extraction and Quantitative Estimation of Lipids

The shoots were cut into small pieces, homogenized in a mortar with quartz sand and acetone and extracted with acetone and light petroleum. A half-saturated NaCl solution was added to the combined extracts, the lipids being extracted in the organic epiphase. The latter was washed with H_2O, dried (Na_2SO_4) and concentrated to 5 ml. These procedures were carried out in dim light.

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The chlorophylls were determined spectrophotometrically in Et_2O using the extinction factors given by Ziegler and Egle.[16] The carotenoids were separated by thin-layer chromatography according to Hager and Bertenrath.[17] The bands were eluted with EtOH and then estimated spectrophotometrically. An estimation factor $E^{1\%}_{1cm} = 2500$ at λ_{max} was used for all carotenoids.

The absorption spectrum of the reduced naphthoquinone vitamin K_1 is not stable. Therefore the concentration of vitamin K_1 cannot be measured by the absorbance change on reduction of the quinone as is done with other lipoquinones. The quantitative determination of vitamin K_1 is best achieved by means of its green fluorescence under u.v. irradiation (254 nm). After two-dimensional TLC on silica gel plates [first dimension: light petroleum–Et_2O (10:1); second dimension after paraffin impregnation of the plate: acetone–H_2O (20:1)] which separates vitamin K_1 from β-carotene and plastoquinone, it is estimated by comparing its light-green fluorescence in u.v. light with that of K_1 spots of a known concentration on a separate plate. This method is specific for vitamin K_1 and allows an accurate estimation of less than 0·5 μg. With some experience and provided that at least two plates are prepared from each lipid extract for the visual K_1 determination, the maximal deviations of this estimation method are less than $\pm 5\%$.

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