Kinetic of Lipoquinone and Pigment Synthesis during Red Light-Induced Thylakoid Formation in Etiolated Barley Seedlings

K. H. Grumbach and H. K. Lichtenthaler

Botanisches Institut der Universität (Pflanzenphysiologie), Karlsruhe

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Red light induces in etioplasts of dark-grown barley seedlings (*Hordeum vulgare* L.) parallel to the formation of chlorophyll an increased synthesis of carotenoids, lipophilic benzoquinones and of vitamin K_1.

1. Among the carotenoids red light initiates an enhanced synthesis of β-carotene, lutein, violaxanthine and neoxanthine whereas the pools of zeaxanthine and antheraxanthine are decreased.
2. The formation of plastoquinone-9, vitamin K_1 and α-tocoquinone is more enhanced than that of α-tocopherol.
3. The red light-induced changes of carotenoid and lipoquinone metabolism are similar in a qualitative sense to those obtained under continuous far-red, white or blue light.
4. In contrast to blue light, red light induces in the first hours of illumination a lower rate of chlorophyll and vitamin K_1 formation. There are also differences in the ratios of the individual pigments and lipoquinones throughout the greening period.

Introduction

The development of the photosynthetic apparatus upon illumination of etiolated plants with different light qualities has been studied in recent years by many authors. The light-induced formation of thylakoid prenyllipids such as lipoquinones, carotenoids and chlorophylls is controlled by the phytochrome system [1–5]. This is also true for the glyco- and galactolipids, which are bound to the photosynthetic membrane [6, 7].

In a previous paper we have shown, that blue light can initiate in etiolated tissues an enhanced thylakoid prenyllipid accumulation and is as effective as white light [8]. It is known from the regulation of plant metabolism, that red light acts differently from blue light. As compared to blue light, red light decreases the photosynthetic activity of acetabularia chloroplasts [9] and stimulates the incorporation of ^{14}C-label in malate, aspartate and glutamate [10], while blue light in turn shows an enhancement of RNA- and protein-synthesis [11–13], promotes photosynthetic glycolate formation [10] and enhances the rates of respiration [14] and photosynthesis [9]. The present paper is a part of an investigation to study, whether the red light-induced development of chloroplasts is similar to that in blue light [8]. Here we report on the formation of the isoprenoid thylakoid lipids: Carotenoids, lipoquinones and chlorophylls.

Requests for reprints should be sent to Prof. Dr. H. K. Lichtenthaler, Botanisches Institut der Universität (Pflanzenphysiologie), *D-7500 Karlsruhe 1*, Kaiserstr. 12.

Methods

The cultivation of barley plants (*Hordeum vulgare* L., Wilferdingen) was performed under defined conditions. These as well as the extraction and estimation procedures are described in a previous paper [8]. The 7-day-old dark-grown barley seedlings were illuminated with continuous red light. The light source consisted of Philips lamps TL 40 W/15 with a 3 mm red plexiglass filter from Röhm GmbH, 61 Darmstadt, FRG, and Hermatherm glass (1000 $mW \times m^{-2}$; $\lambda_{max} = 660$ nm). In each case 150 plants (primary leaf + coleoptile) were extracted. The results represent mean values of several determinations with maximal deviations of $\pm 5\%$.

Results

a. Chlorophylls

Chlorophylls are located together with carotenoids and lipoquinones in the photochemically active thylakoids of chloroplasts [15]. During the red light-induced thylakoid formation the level of chlorophyll a and b increases continuously (Fig. 1). The formation of chlorophyll a precedes that of chlorophyll b as it is known from greening in blue or white light. This results in high values (5, 7) to the a/b ratio, which

	7-d-dark plants	+ red light 6 h	10 h	24 h	48 h
fresh weight [g]	15.4	15.12	15.64	15.74	15.95
dry weight [g]	1.18	1.26	1.29	1.43	1.65
chlorophylls [mg]	—	0.43	1.11	3.60	6.06
carotenoids [μg]	297	340	510	880	1160.
benzoquinones [μg]	193	236	250	308	445
naphthoquinones [μg]	10	11	13	17	21
	7-d-dark plants	+ blue light 6 h	10 h	24 h	48 h
chlorophylls [mg]	—	0.86	1.48	3.97	6.10
carotenoids [μg]	232	301	417	680	1002.
benzoquinones [μg]	184	206	251	347	445
naphthoquinones [μg]	10	12	17	22	29

Table I. Weight and prenyllipid content of 7-day-old etiolated barley seedlings upon illumination with red and blue light. The latter data are taken from a previous paper [8].

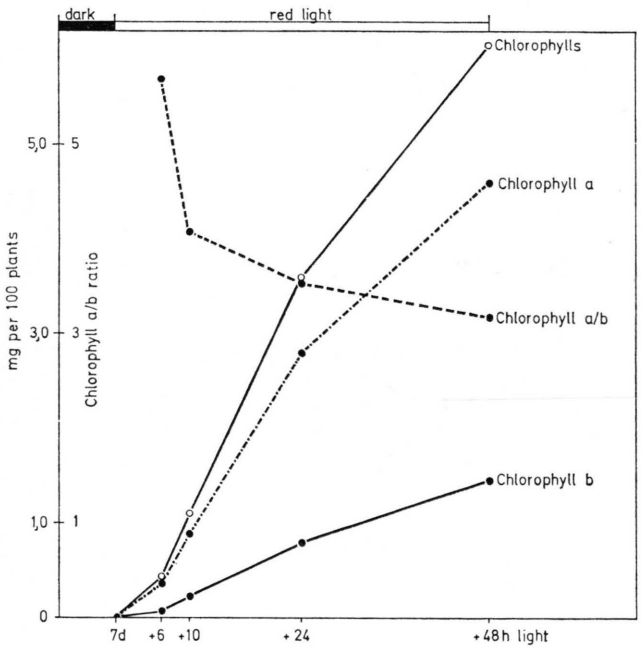

Fig. 1. Chlorophyll content and a/b ratio in 7-day-old barley seedlings during greening in red light.

then decreases with increasing illumination time to a value of about 3 (Fig. 1). In contrast to greening in blue light, which has been tested under the same growth conditions and in a comparable light intensity [8], there are formed much less chlorophylls in the first 24 h of illumination (Table I).

b. Lipoquinones

The isoprenoid benzo- or naphthoquinones of chloroplasts, which are potential electron carriers in photosynthesis, are synthesized in red light parallel to the formation of chlorophylls. Among the lipophilic benzoquinones there is formed a higher portion of plastoquinone-9 (+ plastohydroquinone-9) than α-tocopherol (+ α-tocoquinone) (Fig. 2). By this, the percentage of total plastoquinone-9 increases from 24% (7-day-old dark plants) to 35% of total lipoquinone content after 48 h of red light. A partial photo-oxidation of plastohydroquinone-9 to plastoquinone-9, which has been detected after 6 h of blue and white light greening, can also be seen after 6 h of red light but to a much lower degree (Fig. 2).

The synthesis rate of isoprenoid benzoquinones in red light corresponds almost to that in blue light (Table I). In the first 6 h of red light exposure we observed a distinct higher formation of plastoquinone-9 (+25%) than in blue light. Thereafter the kinetics are nearly the same in both light qualities.

c. Naphthoquinones

The etioplasts of dark-grown barley seedlings contain beside vitamin K_1 a second lipophilic naphthoquinone which chromatographically behaves like desmethylvitamin K_1. This substance is referred to in the text as vitamin "K". The level of vitamin "K", which seems to be a precursor of vitamin K_1 [2], decreases in continuous white light parallel to the augmentation of the vitamin K_1 accumulation. Red light results in an enhanced synthesis of the phylloquinone vitamin K_1 (Fig. 3). In contrast to white light or continuous far-red light [2] we found in red light only a small decrease after 6 h in the level of vitamin "K" followed by a low but constant increase in its pool size. This might be a special red light effect and was not seen in blue or continuous white

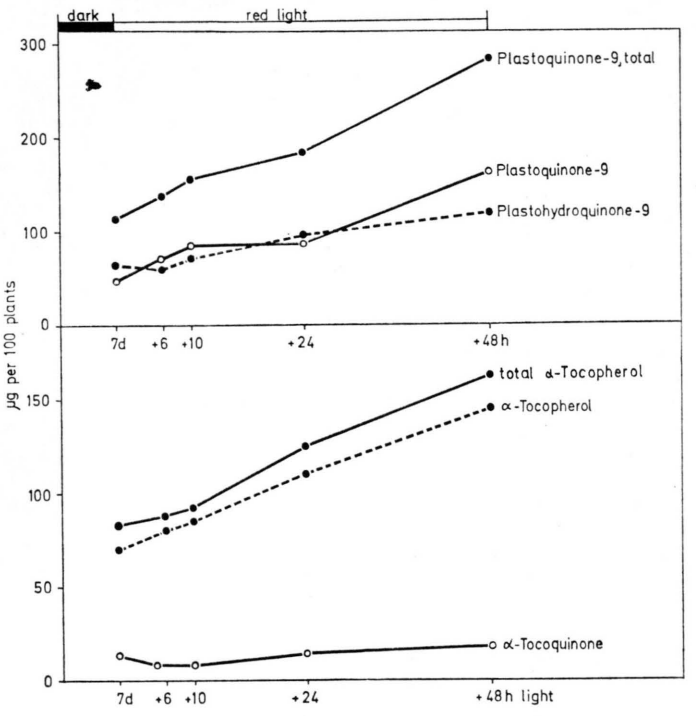

Fig. 2. Formation of benzoquinones in etiolated barley seedlings in red light.

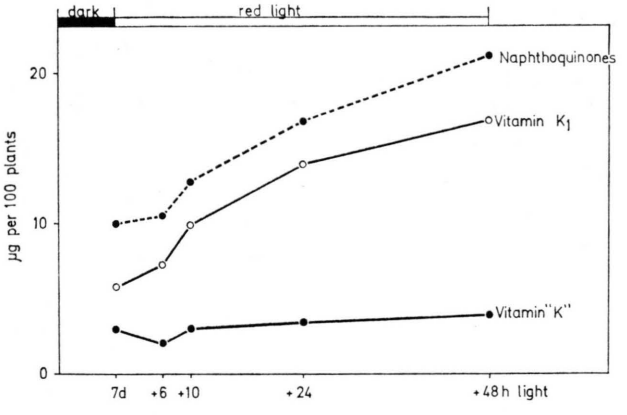

Fig. 3. Formation of naphthoquinones in etiolated barley seedlings in red light.

light. The total amounts of naphthoquinones formed are initially the same in red and blue light with a significant higher level in the blue light-treated plants after 24 and 48 h of illumination (Table I).

d. Carotenoids

Etiolated barley seedlings mainly contain the xanthophylls lutein, violaxanthine, antheraxanthine and zeaxanthine with only low concentrations of β-carotene. During greening in red light the carotenoid synthesis is much enhanced with a particularly strong increase in the β-carotene content ($18\times$) followed by neoxanthine ($9\times$) and lutein ($3\times$). The level of violaxanthine increases at first too but then remains fairly constant (Fig. 4). The concentration of anthera- and zeaxanthine slowly decreases in red light. Concomitantly the percentage composition of total carotenoids from dark-grown seedlings changes considerably (Table II). After 48 h it resembles that of fully green light-grown tissues. Though the kinetics for the accumulation of individual carotenoids in red light are quite similar to those in blue light, there are differences in the caro-

Table II. Changes in the percentage composition (weight %) of carotenoids in 7-day-old etiolated barley seedlings and after 48 h illumination with red or blue light.

	7-d-dark plants	+ 48 h red light	7-d-dark plants	+ 48 h blue light
β-carotene	5.3	25.7	7.8	31.9
lutein	44.0	41.5	44.5	38.2
violaxanthine	23.1	19.7	26.5	23.1
neoxanthine	3.9	10.0	2.5	5.5
antheraxanthine	19.8	2.2	16.3	1.2
zeaxanthine	3.9	0.9	2.4	0.1
carotenoids	100.0	100.0	100.0	100.0

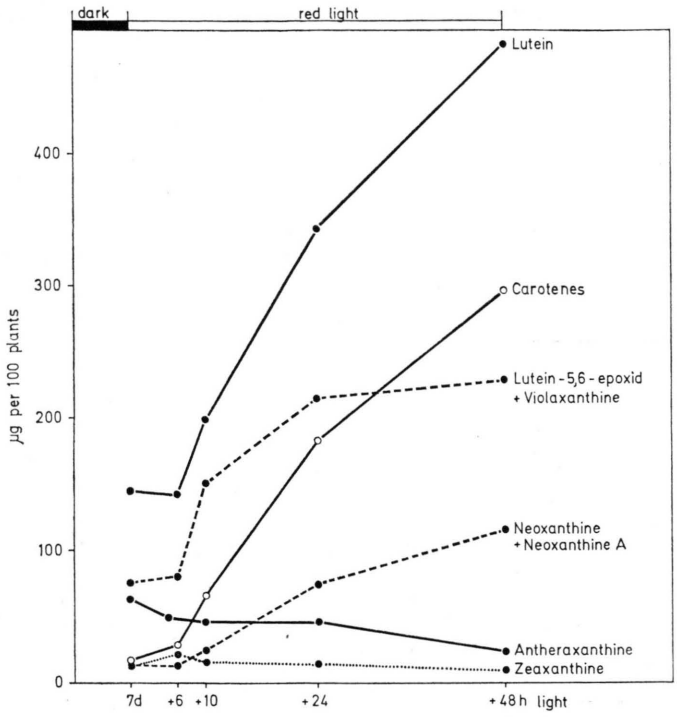

Fig. 4. Carotenoid accumulation in 7-day-old etiolated barley seedlings during greening in continuous red light.

tenoid percentage between red and blue light-treated plants. Thus the latter contain a significant higher percentage of β-carotene (31%) than plants which were grown in red light (25.7%). Correspondingly the values for the ratio xanthophylls to β-carotene are higher in red light-treated plants during all stages of illumination (Table III). In addition red light-treated plants still contain a higher concentration of anthera- and zeaxanthine than blue light-treated plants. This indicates that the process of greening proceeds slower in red than in blue light, though the total amount of carotenoids formed in the light are about the same after 48 h.

Table III. Changes in the lipoquinone and pigment ratios of 7-day-old barley seedlings during greening in continuous red or blue light.

(a+b)	= chlorophylls	x	= xanthophylls			
a	= chlorophyll a	BQ	= benzoquinones			
(x+c)	= carotenoids	PQ-9	= plastoquinone-9			
c	= β-carotene	K_1	= vitamin K_1			

	7-d-dark plants	+6 h	+10 h	+24 h	+48 h light	
x/c	17.8	10.8	6.7	4.4	2.9	red
x/c	11.6	5.8	3.6	2.4	2.1	blue
(a+b)/(x+c)	—	1.3	2.2	4.1	5.2	red
(a+b)/(x+c)	—	2.9	3.6	5.9	6.2	blue
a/K_1	—	42.3	89.0	204.0	266.0	red
a/K_1	—	59.7	70.0	145.0	168.6	blue
a/PQ-9	—	5.4	10.6	32.2	28.3	red
a/PQ-9	—	8.1	9.4	16.6	19.4	blue
BQ/K_1	34	24	32	22	26	red
BQ/K_1	18	17	15	16	15	blue

Discussion

The results of this investigation thus show, that the greening of etiolated tissue in continuous red light follows in principle the same general kinetic as the formation of chloroplasts in white or blue light [8], or in continuous far-red light [2]. In all cases the lipoquinone and carotenoid metabolism is gradually changed upon illumination *via* that of functional chloroplasts from fully green plants. In an induction and reversion experiment with short pulses of red and/or far-red light it has been shown that phytochrome initiates this light-induced change in the carotenoid and lipoquinone metabolism [4, 5]. We thus assume that the red light-induced enhancement of prenyllipid synthesis shown here is also mediated by active phytochrome.

There are, however, certain differences between red and blue light-treated plants concerning the quantitative amount of prenyllipids formed. This

refers in particular to the chlorophyll synthesis, which is lower in the first hours of illumination in the red light-treated plants, but then reaches about the same level as in blue light. From this it appears that the greening process in blue light initially proceeds faster than in red light of a comparable light intensity. The values for the ratio chlorophylls/carotenoids are similar in both light qualities, which indicates that chlorophylls and carotenoids are synthesized in a more or less correlated way independent from the light quality. The higher values for the ratio Chl a/K_1 and Chl a/PQ-9 show (Table III) that the stimulation of the plastoquinone-9- and vitamin K_1-synthesis is not in the same way correlated to chlorophyll synthesis as in blue light. This may be another indication that the greening process proceeds slower in red as compared to blue light. Since plastoquinone-9 and vitamin K_1 are potential electron carriers in photosynthesis, this might also mean that the photosynthetic units are built up in a different way, with eventually less photosynthetic centers on a chlorophyll basis in red than in blue light. This possibility will be tested by further investigations.

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