Light-Induced Accumulation and Stability of Chlorophylls and Chlorophyll-Proteins during Chloroplast Development in Radish Seedlings

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Illumination of 3 day old etiolated radish seedlings with continuous white light results in a progressive accumulation of chlorophyll a and b. Both pigments are bound in a different way to the thylakoid chlorophyll-proteins, which appear parallel to the formation of chlorophylls. By applying the SDS-PAGE method to SDS-digested chloroplasts, it was possible to show that the chloroplasts of radish cotyledons contain the typical chlorophyll proteins LHCP$_{1-3}$, CPa, CPI and CPIa which have been found in other plants. Between LHCP$_1$ and CPI an additional chlorophyll protein is detected with the spectral properties of a LHCP; it is termed here LHCP$_y$.

When the green plants are transferred to continuous darkness, chlorophylls and the chlorophyll-proteins are progressively degraded. At an early stage of greening chlorophyll b is destroyed at a much higher rate in darkness than chlorophyll a, which yields high chlorophyll a/b ratios. This is paralleled by a faster decrease in the level of the corresponding chlorophyll a/b-protein LHCP$_3$ than of CPI. At a later stage of greening, after the end of the logarithmic chlorophyll accumulation, the chlorophylls a and b and also the LHCP$_3$ and CPI are destroyed in continuous darkness at equal rates; the a/b ratios and the LHCP$_3$/CPI ratios are then little different from the light control.

The data indicate that at an early stage of greening the light-harvesting chlorophyll a/b-protein LHCP$_3$ is less stable than the other chlorophyll-proteins (CPI, CPIa, CPa), which contain predominantly chlorophyll a.

The ratio chlorophyll a to β-carotene (a/c ratio) of CPIa, CPI and CPa is about 10, while that of the LHCP$_{1-3}$ is found to be between 150 to 300. We therefore propose using the a/c ratio to define the chlorophyll-proteins which, besides the absorption spectra, is the most suitable parameter.

Introduction

During the light-induced formation of chloroplasts in the leaves of etiolated seedlings, chlorophyll a and b are accumulated parallel to the light-induced formation of photochemically active thylakoids [1 – 4]. The accumulation of chlorophyll a precedes that of chlorophyll b, giving rise to initially high chlorophyll a/b ratios at short illumination times [5 – 7]. With increasing light exposure the chlorophyll a/b ratio decreases to the steady state values of about 3, which, on the average, are higher in sun leaves and high-light plants (3.5 – 4.5) than in shade leaves and low-light plants (2.6 – 3.2) [8 – 11].

Chlorophyll a and b are quantitatively bound to the photochemical active thylakoids [12, 13] in form of chlorophyll-proteins [14, 15]. Polyacrylamide-gel electrophoresis of sodium dodecylsulphate digested chloroplasts (SDS-PAGE) reveals two main chlorophyll proteins, the light-harvesting chlorophyll a/b-protein (LHCP = LHCP$_3$) and the chlorophyll-protein of the pigment system I (CPI), which exhibit different chlorophyll and carotenoid composition [14 – 21]. Besides these, several other minor chlorophyll-proteins have been found more recently, namely LHCP$_1$, LHCP$_2$, CPa and CPIa [18 – 21]. LHCP$_1$ and LHCP$_2$ are regarded as dimers or oligomers of LHCP$_3$, and CPIa as an oligomer of CPI [18]. Chlorophyll b is apparently quantitatively located in the light-harvesting chlorophyll a/b-protein LHCP, which exhibits low chlorophyll a/b ratios of 1.0 to 1.5 [14, 21]. The two chlorophyll-proteins of photosystem I – the P 700-containing photochem-

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Abbreviations: a/b, ratio chlorophyll a/b; CPI, chlorophyll-protein I = P 700 chlorophyll a-protein; CPIa, chlorophyll-protein I a; CPa, chlorophyll-protein a; FP, free pigments (chlorophylls and carotenoids); LHCP$_{1-3}$, light-harvesting chlorophyll a/b-protein 1,2 and 3; PAGE, polyacrylamide-gel electrophoresis; SDS, sodium dodecylsulphate; Tris, tris (hydroxymethyl)-aminomethan; v/v, volume per volume; w/v, weight per volume.

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ically active CPI and CPIa [22] – in turn mainly consist of chlorophyll a with high a/b ratios above 7 [14, 18, 21]. CPa is assumed to represent the reaction center of photosystem 2 [18, 22–25].

Differences in chlorophyll a/b ratios as found between different plants or the same plants cultivated under different growth conditions, reflect differences in the level of the two main chlorophyll-proteins LHCP and CPI. Chloroplasts of low-light radish and barley plants possess low a/b ratios and a higher level of LHCP (higher values for the ratio LHCP/CPI) than the chloroplasts from high-light plants, which exhibit higher chlorophyll a/b ratios and less LHCP on a CPI basis [26].

As early as 1938, Seybold and Egle showed that the chlorophyll a/b ratios of young seedlings which had greened only for a few hours increased very much during a subsequent dark period [5]. In etiolated *Phaseolus* seedlings illuminated for 24 h the a/b value of 3.8 at the end of the light period increased to 15 within 120 h of continuous darkness, due to a faster destruction of chlorophyll b than a. In Triticum and Lipidium seedlings which had been illuminated for 10 h the a/b ratio increased in the dark period (72 and 53 h) from 4.1 and 3.4 to 7.7 and 6.7 respectively [5]. In 1971 similar results were reported for pea seedlings [6]. When illuminated with white light for a short period (1 h), the chlorophyll a/b ratio increased in the subsequent dark period (6 h) from 14 to 50. Pea plants greened for longer periods (6.5 and 16 h), before being returned to darkness showed, however, a greater stability in their chlorophyll a/b ratios; the increase in the a/b ratio in continuous darkness then occurred later and was less pronounced than in the seedlings illuminated only for a short time [6].

These older results [5, 6] clearly show that during greening chlorophyll b is degraded more rapidly than chlorophyll a. They also indicate that the instability of the chlorophyll b pool in darkness decreases with increasing illumination time. Unfortunately longer illumination times have not been studied. Therefore it is not clear whether and when the chlorophyll b pool becomes as stable as the chlorophyll a pool.

In view of the binding of chlorophyll b to the light-harvesting chlorophyll a/b protein LHCP, the great changes in chlorophyll a/b ratios in darkness indicate that in the first hours of greening the LHCP is either not yet stable or less stabilized than the CPI. To prove this, a comparative study has been made of the variation in the chlorophyll a/b ratios as well as in the appearance and stability of the different chlorophyll-proteins during the logarithmic and steady state phase of chlorophyll accumulation in cotyledons of radish seedlings.

Materials and Methods

Radish seedlings (*Raphanus sativus* L. var. Saxa Treib) were cultivated on a modified van der Crone nutrient solution [27] in the dark for 3 days at 22 °C and 65% relative humidity. The etiolated plants were then illuminated with continuous white light (Tungsram lamps 65 W; 3000 lux = 6.5 W · m^{-2}).

Chlorophylls were extracted from the leaf material (50 to 100 cotyledons) with acetone and light petrol and then measured in diethyl ether [28]. Carotenoids were determined by high performance liquid chromatography [26].

The chloroplasts of radish cotyledons were isolated in the isolation medium consisting of 0.1 M potassium phosphate buffer (pH 7.5), 0.35 M sorbit, 0.01 M sodium-EDTA, 0.005 M $MgCl_2$, and 0.02 M mercaptoethanol. 150 to 200 cotyledons (~ 20 to 25 g fresh weight) were ground for 2 min in 25 ml cold isolation buffer (4 °C; Waring blendor), the filtrate (fine nylon cloth) centrifuged for 20 min at $5000 \times g$ and the chloroplasts in the sediment osmotically broken by suspension in bi-distilled ice-cold water and centrifuged at $10\,000 \times g$ (20 min). The washing was repeated twice and the final membrane sediment resuspended in a little ice-cold water. The determination of total chlorophyll was performed after the method of Arnon [30]. The membrane fraction was then solubilized in a Tris-HCl-buffer (50 mM; pH 6.8) containing 1% mercaptoethanol (v/v) 10% glycerol (v/v) and 0.55% SDS (w/v). The solution contained about 0.15 μM chlorophyll per ml (weight ratio: chlorophyll/SDS = 1:40). After an incubation time of 5 to 10 min the SDS-membrane solution was centrifuged at $10\,000 \times g$ for 5 min to remove small amounts of undissolved membrane material, which occasionally may be present.

Disk electrophoresis was performed in gel cylinders (length 45 mm, inner diameter 6 mm) at 4 °C applying approximately 100 μl SDS-solubilized membrane solution (~ 135 μg chlorophyll) following the method of Laemmli [31]. The final concentration of the stacking gel (length 5 mm in the tube) was 3% acrylamide, 0.125 M Tris-HCl (pH 6.8) and 0.1% SDS. The separation gel consisted of 10% acrylamide 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS. The electrode buffer, a Tris (0.025 M) -glycine (0.192 M) -buffer (pH 8.3), contained 0.1% SDS. The polyacrylamide gels were prepared from a stock solution of 30% acrylamide + 0.8% N,N′-bis-methylene acrylamide (w/v). Polymerisation was performed by addition of tetramethylethylenediamine (TEMED; 0.025 volume %) and of ammonium persulphate (final concentration stacking gel 0.15%, separation gel 0.05%). The separation was first carried out at an initial current of 1 mA for about 20 min; when the chlorophyll-proteins had moved from the stacking to the separation gel, the current was increased to 2 mA per tube. The total resolving time was 90 min.

The relative amounts of the different chlorophyll-protein bands were determined by scannings at 670 nm, 663 and 653 nm (RFT scanning densitometer). Protein bands were visualized by fixation in a solution of 1% amido black in 7% acetic acid. The absorption spectra of the different chlorophyll-proteins were measured a) in the focussed gel bands in a special cuvette constructed for this purpose in a Shimadzu spectrophotometer and also b) after elution of the individual chlorophyll-proteins from the gels with a 0.01 M Tris-HCl buffer, pH 8.

The results of chlorophyll accumulation represent mean values based on 5 different cultivations. As to the chlorophyll-proteins, the data are based on 3 to 5 cultivations with several electrophoretic runs in each case.

Results

Chlorophyll accumulation

Upon illumination of 3-day old etiolated *Raphanus* seedlings, the cotyledons green and exhibit a continuous growth up to the 5th day of illumination. A rapid and large chlorophyll accumulation proceeds up to the 3rd day of illumination (Fig. 1); this is regarded here as the "logarithmic phase" of the chlorophyll accumulation. From the 3rd to the 9th day there is little chlorophyll accumulation; this stage is referred to as "steady state phase" of chlorophyll formation in radish cotyledons. After 8 days of continuous light there occurs on initially slow but continuous degradation of chlorophylls, which is due to cotyledon senescence; this process is paralleled by the development of the primary leaves which overtake photosynthetic production.

Darkening of younger and older plants results in a degradation of chlorophylls. The initial rate of destruction within the first 2 or 3 days appears to be similar in all plants (Fig. 1). Re-illumination after 2 days of darkness stops the chlorophyll decrease and causes reaccumulation of chlorophylls. Within two days of reillumination, the original chlorophyll level (before darkening) is reached or exceeded in the younger plants; the older plants react less, the re-increase in the chlorophyll a + b level is retarded and proceeds more slowly.

Ratio chlorophyll a/b

The values for the ratio chlorophyll a/b are higher in the illuminated plants during the active chlorophyll accumulation phase (3.5) and then decrease to values of 3.0 to 2.9 during the "steady state" phase of chlorophyll formation (Fig. 2). The first hours of illumination, where a/b ratios of 50 to 20 can be found, are not included in the present study. When the younger plants (1 to 3 days of illumination), which are in or at the end of the "logarithmic phase" of chlorophyll accumulation, are darkened, chlorophyll is broken down and the a/b ratio rises to values of about 5. This rise in the a/b ratio is much faster in the plants after 1 day than after 3 days of illumination (Fig. 2a). These data show that chlorophyll b is degraded faster in continuous darkness than chlorophyll a, a process that is slowed down with increasing illumination time. Upon reillumination of the darkened younger seedlings, the chlorophyll a/b ratio decreases within 2 days to about the level of the control plants kept in continuous white light.

In older plants illuminated for 4, 5 or more days, there is only a small but significant rise in the a/b ratios within a 2 day dark period (Fig. 2b), which is not further increased even at a longer dark time of 5 days. Chlorophyll a and b are broken down in darkness at nearly the same rate. As in younger plants, reillumination reduces these slightly increased a/b values to the levels of the plants kept in continuous light.

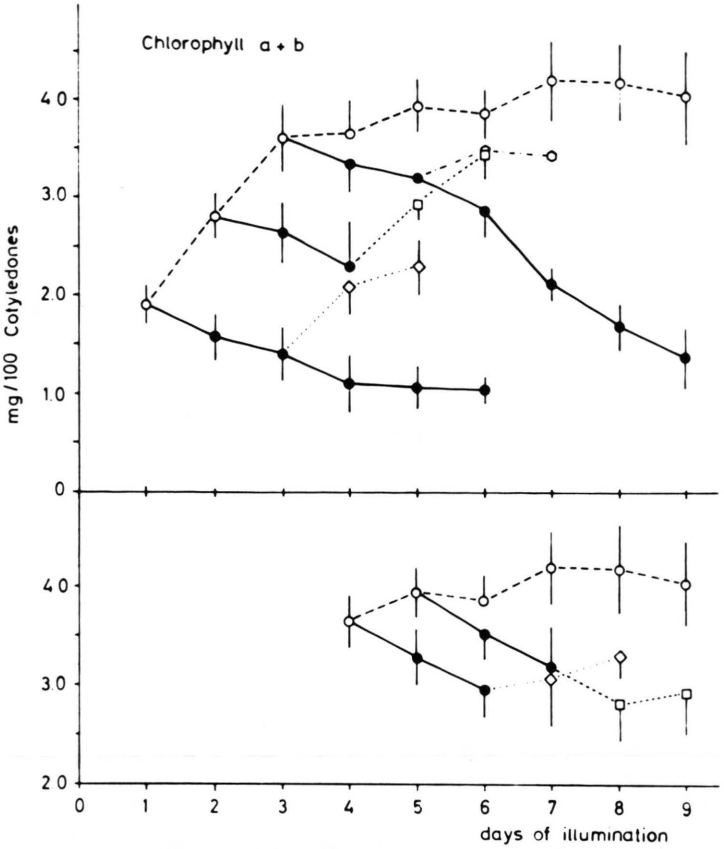

Fig. 1. Accumulation of chlorophyll a and b in the cotyledons of 3 day old etiolated radish seedlings in continuous light (○- - -○); degradation of chlorophyll a + b in continuous darkness (●—●) after different illumination times and re-accumulation of chlorophyll after re-illumination of the darkened plants (□ · · · □). a) younger plants, b) older plants.

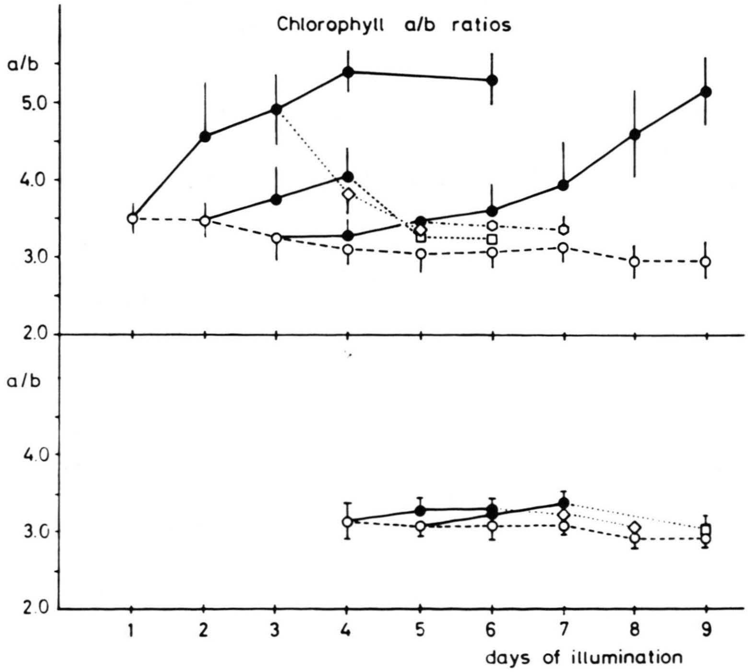

Fig. 2. Development of chlorophyll a/b ratios in the cotyledons of 3 d old etiolated radish seedlings upon illumination with white light (○- - -○). Increase of a/b ratios in plants darkened after 1 to 5 d of illumination (●——●) and decrease of the a/b ratio upon re-illumination of the darkened plants (□ · · · □). a) younger plants with large changes and b) older plants with small changes.

Chlorophyll-proteins

By SDS-PAGE of SDS-digested radish chloroplasts, 7 chlorophyll-proteins in different relative amounts are found (Fig. 3). Besides the chlorophyll-proteins, other proteins are presented and these can be visualized with amido black (Fig. 4).

a) General characteristics

The main component is the light-harvesting chlorophyll a/b-protein $LHCP_3$, which is characterized by a low value for the ratio chlorophyll a/b of 1.3 (Table I) and an absorption spectrum with maxima at 672 and 653 nm (Fig. 6) when measured directly on the gel. In the eluates these two maxima usually shift slightly towards a shorter wavelength of 671 or 670 and 652 nm. Similar spectra are found for $LHCP_1$ (a/b = 1.1), $LHCP_2$ (a/b = 1.3) and for the newly detected $LHCP_y$ (Fig. 6). $LHCP_1$ often appears as a double band. CPI and CPIa, the two chlorophyll-proteins related to photosystem I possess identical spectra (Figs. 7 and 8), which are different from those of the LHCP's. The 653 nm peak, indicating chlorophyll b, is absent in the CPI; there is also less absorption in the carotenoid region between 500–420 nm. The absorption maxima in the red region is shifted towards longer wavelengths (678 nm on the gel; 673–674 nm in the eluates), indicating

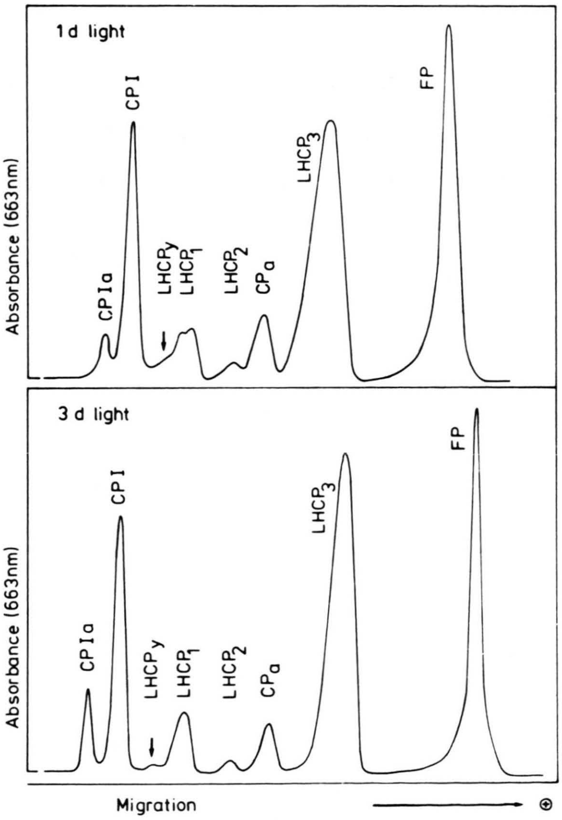

Fig. 3. Densitometer scans at 663 nm of the chlorophyll proteins separated by SDS gel electrophoresis showing differnt chlorophyll proteins. The free pigment zone FP consists of non-protein bound chlorophylls and carotenoids. a) 1 d after illumination of the etiolated seedlings, b) after 3 d of illumination.

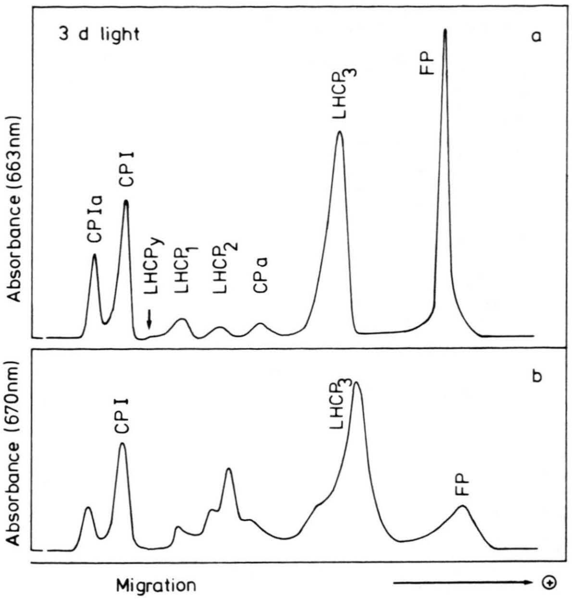

Fig. 4. Comparison of the densitometer scans of the chlorophyll proteins isolated from 3 d illuminated radish chloroplasts by SDS-PAGE a) before and b) after staining with amido black, which reveals additional protein bands.

Table I. Weight ratios of chlorophylls and carotenoids in different chlorophyll proteins isolated from SDS-digested chloroplasts of radish seedlings illuminated for 3 days. The different chlorophyll protein bands were cut out from the gel, eluted with a diluted Tris-HCl buffer (0.01 M; pH 8) and the pigments extracted without further purification. c = β-carotene; x = xanthophylls; x + c = total carotenoids.

Pigment ratio	CPIa	CPI	CPa	$LHCP_3$	FP	Whole chloroplasts
a/b	9.1	7.3	3.6	1.3	3	3.2
a/c	10.7	10.0	11.9	290	15.1	18.5
c/x	3.7	1.4	0.4	0.03	0.26	0.5
$\frac{a+b}{x+c}$	9.3	6.6	5.1	14.5	4.2	8.2

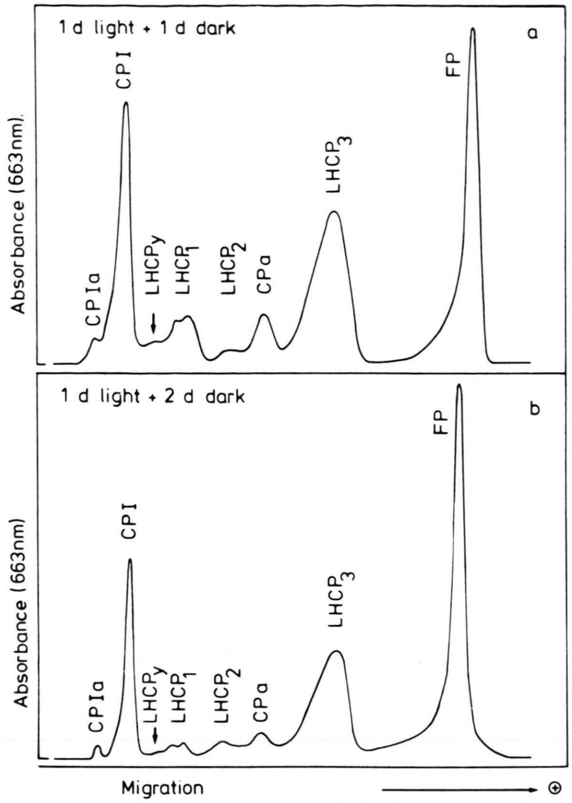

Fig. 5. Densitometer scans of the chlorophyll proteins of radish chloroplasts isolated by SDS-PAGE from plants darkened for 1 d (a) or 2 d (b) after an initial illumination time of 1 d.

different chlorophyll a forms than in the LHCP's. The differences in light absorption between CPI and LHCP are shown in Fig. 7. CPI and CPIa are characterized not only by high chlorophyll a/b ratios (7 to 9), but also by a high β-carotene and low xanthophyll content, as seen from the higher values for the ratio of β-carotene to xanthophyll (Table I). The CPIa is certainly purer than the CPI, which may partly be contaminated by some LHCP. The very low c/x value of 0.03 indicates that the LHCP$_3$ mainly consists of xanthophylls and contains β-carotene only in trace amounts (Table I).

The chlorophyll a to β-carotene ratio (a/c) is a much better parameter to characterize the different chlorophyll proteins than the chlorophyll a/b ratios. The a/c values for CPIa and CPI are low (10.7 and 10) as compared to those of the LHCP's (170–300). It is of interest that the CPa, which is thought to either represent or to be associated with the reaction center of photosystem II [18, 22–25], has a similar a/c ratio to CPI and CPIa; in all three cases there is one mole of β-carotene per 5 to 6 mol of chlorophyll a. The spectrum of the CPa (Fig. 8) is different from that of the LHCP's and the CPI's; it does not exhibit the chlorophyll b shoulder at 653 nm, though after pigment extraction some chlorophyll b may be found in the non-purified CPa-fraction. Since the CPa is only a small fraction appearing on the electrophoretogram between the LHCP$_2$ and the large LHCP$_3$ band, it is contaminated by LHCP's after only one electrophoretic run. Therefore the chlorophyll a/b ratio of the pure CPa is higher than the range of 3 to 3.6 usually found. In two relatively pure preparations a/b values of 5 to 6 were measured. The free pigment zone, which contains non-protein bound chlorophylls and carotenoids, consists of two bands, which overlap. The yellow carotenoid band migrates further than the chlorophyll band. Its absorption spectrum shows little chlorophyll and contains three maxima in the blue region, which are typical for carotenoids (Fig. 9). The free chlorophyll band, in turn, does not exhibit these carotenoid maxima. The free pigment zone of SDS-digested chloroplasts of illuminated plants contains both chlorophylls in about the same ratio as whole chloro-

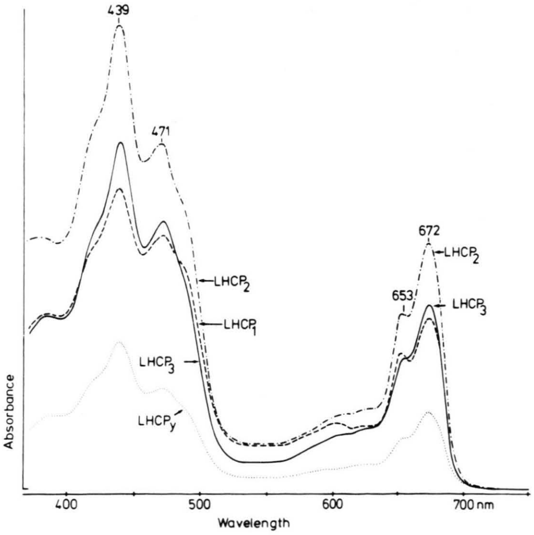

Fig. 6. Absorption spectra of the four light-harvesting chlorophyll a/b-proteins (LHCP$_{1-3}$ and LHCP$_y$) showing the typical double maxima in the red region at 672 and 653 nm, which derive from chlorophyll a and chlorophyll b respectively. The maximum at 471 is mainly due to xanthophylls. The LHCP's were isolated from chloroplasts of radish seedlings illuminated for 3 d.

plasts. The ratio for c/x (β-carotene/xanthophylls) is, however, lower than in whole chloroplasts indicating that this band contains a higher proportion of xanthophylls than β-carotene (Table I). The lower value for chlorophylls/carotenoids (a + b/x + c) shows that the percentage of carotenoids found in this pigment zone is lower than that of chlorophylls. From the different pigment ratios shown in Table I one may conclude that the carotenoids are more tightly bound to the chlorophyll proteins than chlorophylls, and that β-carotene is more stronger associated with the CPI, CPIa and CPa than the xanthophylls with the LHCP's.

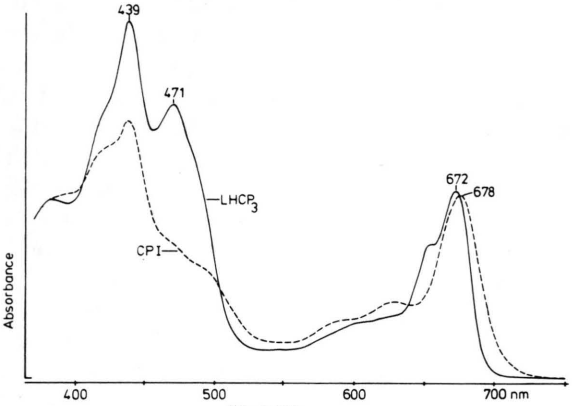

Fig. 7. Comparison of the absorption spectra of two main chlorophyll proteins CPI and $LHCP_3$, which, due to their different pigment composition, possess very different spectral characteristics. Isolation from SDS-digested chloroplasts of *Raphanus* seedlings after 3 d light exposure.

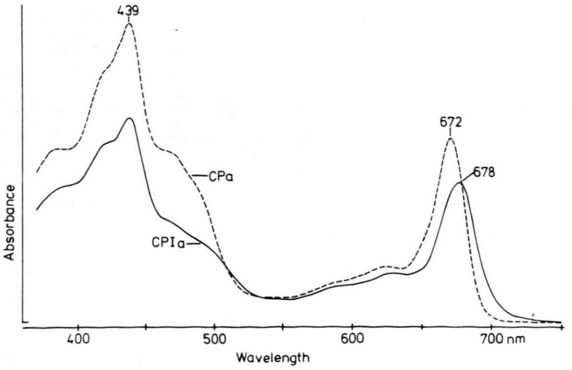

Fig. 8. Absorption spectrum of the chlorophyll-protein CPa, a presumptive component of the reaction center of photosystem II [22–25], as compared to that of CPIa, which is related to photosystem I [22].

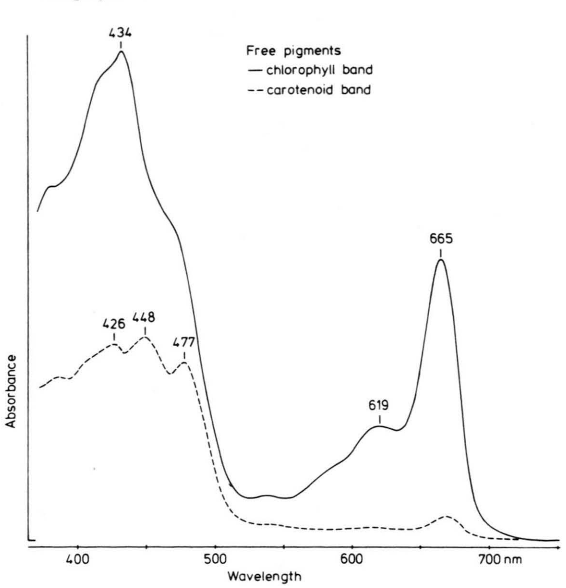

Fig. 9. Absorption spectra of the two partly overlapping sub-bands of the free pigment zone FP: the green chlorophyll band and the yellow carotenoid band.

b) Changes during greening

Parallel to the chlorophyll formation there occurs a continuous accumulation of chlorophyll proteins during greening and growth of the radish cotyledons. Between etiolated radish seedlings that have greened for 24 h in white light and those illuminated for 3 or 5 days, there exist differences in the relative levels of the individual chlorophyll proteins. After 24 h light, the level of the CPIa is very low, but then increases to 5.8% of the total chlorophyll content on the third day of light exposure (Table II). The relative proportion of CPI also increases, while that of the CPa decreases. Though the levels of the CPa are smaller and difficult to measure (large variation), this decrease in CPa with increasing illumination has been found in all cases. Whether there occur changes in the level of the newly found $LHCP_y$ during prolonged greening cannot be judged from the present study, due to the very low amounts of this pigment-protein.

The sum of the proportions of the three $LHCP_{1-3}$ does not change prolonged illumination; the relative level of $LHCP_3$, however, decreases, while those of $LHCP_1$ and $LHCP_2$ increase (Table II). The relative amounts of chlorophyll in the free pigment fraction are distinctly higher after 1 d than after 3 or 5 d of illumination. These changes during greening and

Tabelle II. Percentage distribution of chlorophylls in the different chlorophyll proteins isolated from SDS-digested chloroplasts of radish cotyledons (with standard deviation). The radish plants were grown for 3 days in the dark and then illuminated with continuous white light (3000 lux = 6.5 W · m^{-2}) for 1 to 5 days (a). Some plants were transferred to darkness after 1 day (b) or after 4 days of illumination (c). a) mean values of at least 6 determinations from 3 cultivations; b) and c) mean values of 3 determinations from 3 cultivations.

	CPIa	CPI	CPa	LHCPy	LHCP$_1$	LHCP$_2$	LHCP$_3$	FP	LHCP$_{1-3}$
a) 1 d light	1.3 ± 0.2	13.0 ± 0.7	4.0 ± 1.4	< 1	3.7 ± 1	1.3 ± 0.5	42.8 ± 0.6	32.9 ± 3.0	47.8
3 d light	5.8 ± 1.3	13.6 ± 1.8	3.9 ± 0.8	< 1	4.7 ± 1	1.7 ± 0.7	39.7 ± 1.2	29.6 ± 2.5	46.1
5 d light	5.6 ± 0.4	15.7 ± 1.6	2.0 ± 1.2	∼ 1	7.3 ± 1	2.1 ± 1.0	38.5 ± 1.4	27.8 ± 1.7	47.9
b) 1 d light + 1 d dark	1.7 ± 0.3	16.0 ± 1.0	3.4 ± 1.5	< 1	3.7 ± 1	1.3 ± 0.3	27.9 ± 3.1	44.2 ± 2	32.9
1 d light + 2 d dark	2.2 ± 0.3	15.6 ± 0.8	4.3 ± 1.2	< 1	2.6 ± 1	2.1 ± 0.6	28.9 ± 2.0	43.3 ± 2	33.6
c) 4 d light + 1 d dark	4.7 ± 0.6	15.1 ± 1.3	2.2 ± 1.2	∼ 1	6.9 ± 1	1.8 ± 0.6	39.1 ± 1.0	29.2 ± 2.3	47.8
4 d light + 2 d dark	5.7 ± 0.4	15.3 ± 1.2	2.1 ± 0.6	∼ 1	7.9 ± 1	1.8 ± 0.9	40.1 ± 0.6	26.1 ± 2.3	49.8

development in the relative proportions are best seen in the ratios of LHCP's to CP's (Table III). The ratio LHCP$_{1-3}$/CPI decreases parallel to the increase in the CPI proportion. Because of the marked increase in the CPIa level, the ratio LHCP$_{1-3}$/CPI + Ia decreases to a high extent. This is also the case for the ratio LHCP$_3$/CPI.

c) Changes in prolonged darkness

When younger radish plants, illuminated for 24 h, are transferred to continuous darkness for 1 or 2 d, chlorophylls and chlorophyll-proteins are gradually degraded. Preferences in the decomposition of certain chlorophyll-proteins give rise to a change in the relative proportions of the remaining chlorophyll-proteins as compared to the end of the light period and to the plants kept longer in light.

There occurs a drastic decrease in the relative amounts of the LHCP$_3$, with a concomitant increase in the relative level of the FP zone (Fig. 5a; Table II). The FP spectrum then also shows a distinct chlorophyll b shoulder near 646 nm. The percentage of LHCP$_1$ also decreases to some extent, while the relative proportions of the other pigment proteins CPI, CPIa, CPa and LHCP$_2$ are increased with respect to the starting percentage distribution at the end of the 24 h light period. These results show that in younger radish plants illuminated for a short time, the chlorophylls of the LHCP$_3$ are degraded at a much higher rate than those of the other chlorophyll-proteins. This preferential degradation of LHCP$_3$ can also be seen in the values for the ratios LHCP's to CPI and CPIa, which in darkness decrease considerably as compared to the light control (Table III).

Table III. Weight ratios of the light harvesting chlorophyll a/b proteins (LHCP's) to the chlorophyll proteins of photosystem I (CPI and CPIa) in chloroplasts of radish seedlings of different ages. a) The 3 d old etiolated plants were illuminated for 1 to 6 d with continuous white light (3000 lux = 6.5 W · m^{-2}); b) after 1 d and 4 d of illumination the plants were placed into darkness for 1 or 2 days (arrows).

ratio		1 d		2 d		3 d	4 d		5 d		6 d
$\frac{LHCP_{1-3}}{CPI}$:	a)	3.68	↘	3.51		3.39	3.2	↘	3.05		3.13
	b)			2.06	→	2.15			3.17	→	3.25
$\frac{LHCP_{1-3}}{CPI + Ia}$:	a)	3.34	↘	2.91		2.38	2.43	↘	2.25		2.44
	b)			1.76	→	1.88			2.41	→	2.37
$\frac{LHCP_3}{CPI}$:	a)	3.29	↘	3.3		2.92	2.65	↘	2.45		2.5
	b)			1.74	→	1.85			2.59	→	2.62

In older plants which have been illuminated for a longer period and which are in the "steady state" phase of their chlorophyll accumulation, the LHCP$_3$ is not as sensitive to destruction in the subsequent dark period than in the younger plants. It is then as "stable" or "unstable" as the other chlorophyll-proteins. Upon darkening of radish plants illuminated for 4 d, the relative percentages of the chlorophyll-proteins are maintained more of less at the same levels as in the light controls (Fig. 4b; Table II). There are also no significant changes in the ratio of LHCP's to CPI and CPIa (Table III).

The percentage distribution of chlorophylls as given in Table II is based on the densitometer scans at 663 nm. This wavelength was chosen because it is most suitable for a simultaneous registration of the LHCP's and the different CP's. Registration at shorter wavelengths would cause an increase in the chlorophyll b-containing LHCP but decrease the relative proportion of the CPI (Fig. 10). Wavelengths above 663 nm, in turn, would favour the CP's.

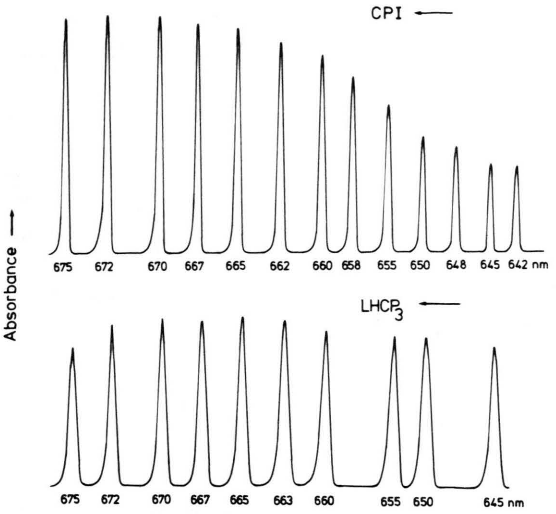

Fig. 10. Densitometer scans of CPI and $LHCP_3$ at different wavelengths showing the dependence of the absorbance height on the applied wavelength.

Discussion

The results of this comparative study demonstrate that the well-known rise in chlorophyll a/b ratios upon darkening of not yet fully greened etiolated plants illuminated for a short time [5, 6] is due to an instability of the main light harvesting chlorophyll a/b-protein $LHCP_3$. This relative instability of the $LHCP_3$ disappears with increasing illumination. When the chlorophyll accumulation in the radish cotyledons is finished ("steady state" phase), the $LHCP_3$ is degraded at the same rate as the other chlorophyll-proteins. Correspondingly the chlorophyll a/b ratios then show little increase in darkness. What causes this initial instability of the $LHCP_3$ is not known. Since chlorophyll a accumulation precedes that of chlorophyll b, as seen by the initially higher chlorophyll a/b ratios [1, 5, 6], which decrease with increasing illumination, one may assume that the light harvesting chlorophyll a/b protein is not yet fully equipped with chlorophyll b.

One has to consider, however, the fact that the LHCP also contains xanthophylls [14, 21, 32 and Table I]. The accumulation of xanthophylls, in turn, lags behind that of chlorophyll a and b, while β-carotene – the main carotenoid of the more stable CPI, CPIa and CPa – is accumulated at a faster rate than xanthophylls upon illumination of etiolated seedlings [1, 33]. From this it is concluded that the absence of the usual amounts of xanthophylls may be another reason for the relative instability of the $LHCP_3$ during the first hours and days of illumination of etiolated seedlings.

Upon darkening of the younger and the older radish plants, breakdown of chlorophylls is correlated with a degradation of the chlorophyll-proteins, the total level of which decreases with increasing darkness. This degradation, which must be seen in connection with thylakoid turnover, is not visible in continuous light, which compensates for the breakdown and keeps chlorophyll and chlorophyll-proteins at a more or less constant "steady-state" level. The rate of chlorophyll destruction in the first 2 or 3 days of darkness is fairly similar, no matter how much chlorophyll has already been accumulated in the cotyledons. This observation indicates that the enzymes for chlorophyll degradation are apparently present before darkening, their synthesis and activity seems not to be enhanced by prolonged darkness. The degradation of chlorophyll amounts to about 10% per 24 h in the older plants and may be regarded as the daily turnover rate.

The question whether the protein of chlorophyll-proteins is degraded at the same rate as the chlorophylls, cannot be answered from the present study. Because of the many other proteins between the chlorophyll-proteins, which show up after staining with amido black (Fig. 4), this would require additional purification steps. From the observation that the total amount of protein per chloroplast sediment prepared from the same number of cotyledons decreases continuously with increasing darkness, it can be assumed that the protein part of the chlorophyll-proteins is degraded too, together with the pigments.

The absorption spectra and the pigment analysis indicate that the various chlorophyll-proteins have a different chemical composition and function. Chlorophyll b and xanthophylls, which are located in the LHCP's, play the role of accessory pigments. CPI is closely related to photosystem I, contains P 700 and is photochemically active [14, 18], which is also assumed for the CPIa [22]. CPa, in turn, is regarded as the reaction center of the photosystem II [12, 22–25]. All three chlorophyll a-proteins (CPI, CPIa and CPa) possess high a/b ratios (Table I). The similarly high level of 1 β-carotene per 5 to 6 chlorophyll a in the CPI, CPIa and in the CPa as shown here, must be seen in relation to a possible role of β-carotene as a quencher of excitation energy in the photosynthetic reaction centers [34–38] of both photosystems. A similar quencher function within the chlorophyll proteins CPI and CPa has also been postulated for the phylloquinone K_1 [21, 39].

Since the LHCP's contain the xanthophylls but only trace amounts of β-carotene, a contamination of the CPI, CPIa and CPa with some LHCP does not affect their β-carotene level; correspondingly such a contamination has much less influence on the ratio chlorophyll a to β-carotene a/c than on the ratio chlorophyll a/b. We therefore using the a/c ratio as an additional and better parameter to define the different chlorophyll-proteins than the ratio chlorophyll a/b.

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