THE CHLOROPHYLL–PROTEIN COMPLEXES OF THE THYLAKOID IN GREENING PLASTIDS OF *PHASEOLUS VULGARIS*

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1. Introduction

The interest placed on the chlorophyll (chl)–protein complexes separated by sodium dodecyl-sulfate (SDS)–acrylamide gel electrophoresis of SDS-solubilized thylakoids [1,2] has been recently renewed with the application of improved methods which enable the detection of new chl–protein complexes, accounting for ⩽ 90% of the thylakoid chl [3–5].

The two chl–protein complexes resolved with the earlier methods (the *P*-700 chl *a*-rich protein complex I (or CPI), assumed to originate from the reaction center of photosystem I(PS-I), and the chl *a* + *b*–protein complex II (or CPII), considered to represent the antenna chl of PS-II, or of PS-I and PS-II [2,6–8]) accounted for 70% of the thylakoid chl [2]. The distribution of chl between these two complexes was found to depend on the developmental stage of the plastid [9], CPII representing the major component of granal membranes and CPI of stroma lamellae [10–12].

In the agranal protochloroplasts (the chl *b*-less plastids of etiolated leaves exposed to periodic light–dark cycles) CPII could not be detected and ⩽ 30% of the primary thylakoid chl could be isolated bound on CPI, the rest running as free pigment [9]. These plastids, however, are very active in both PS-I and PS-II reactions, much more than the mature green chloroplasts (on a chl basis), and they are considered to contain mainly reaction center chl [8,13,14]. Assuming that CPI originates from PS-I, and considering that CPII does not represent the reaction center of PS-II but only the light harvesting antennae of PS-II (or possibly of PS-I as well), we always considered that an unstable chl–protein complex originating from the reaction center of PS-II was present but could not be detected by the methods available.

The milder procedures used recently, enable the detection of ⩽ 6 chl–protein complexes from thylakoids of mature green chloroplasts: CPIa (an oligomeric form of CPI), CPI, LHCP[1] and LHCP[2] (oligomeric forms of LHCP[3]), LHCP[3] (believed to be identical to CPII) and CPa [4]. This last complex, the CPa, is considered to originate from the reaction center of PS-II [3,4].

Applying these procedures to the SDS-solubilized primary thylakoids, apart from CPI, an additional complex can be detected, running between CPI and the free pigment band, at the position where CPa runs [4] and having similar absorption spectrum as that reported for CPa [4] or complex IV [3]. This complex accounts for ⩽ 40% of the primary thylakoid chl *a*, while it represents only a minor constituent of the thylakoids of mature green chloroplasts. The light harvesting complexes are detectable after prolonged exposure to periodic light, and they are formed in excess after transfer of the plants to continuous light. The light harvesting complexes represent the major components of isolated granal thylakoids.

2. Methods

We used 6–7 day etiolated *Phaseolus vulgaris* plants handled and grown as in [15]. They were exposed to periodic light–dark cycles (2 min white light–98 min dark) then transferred to continuous

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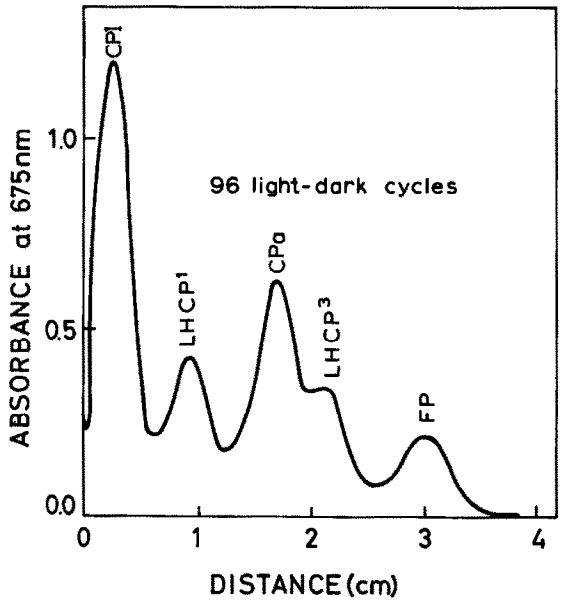
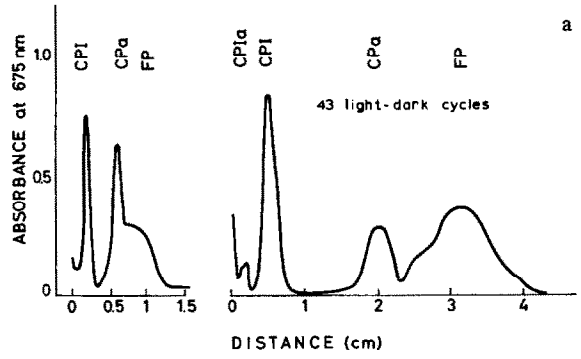
light [15]. Plastids were isolated as pellets at 3000 × *g* (leaves exposed to periodic light) or 1000 × *g* (mature green leaves) for 10 min, after homogenization of 4 g fresh wt leaves at a time with 40 ml buffer (0.3 M sucrose–0.05 M phosphate–0.01 M KCl, pH 7.2) and were washed twice with ice-cold distilled water. The thylakoid pellet (recovered at 15 000 × *g* for 15 min) was dissolved just before the electrophoretic run with ice cold 0.3 M Tris–HCl (pH 8.8)–10% glycerol–1% SDS and SDS/chl ratio of 10 [4]. For some experiments this ratio varied. The electrophoresis was done according to [4] in the cold room with the following modifications: the stacking gel stacked at pH 6.1 (buffer of [16]) and the resolving gel at pH 8.64 [16]. The *N,N,N'N'*-tetramethylenediamine in the gels was 0.005% (v/v). The running buffer was at pH 9.5 [4,16]. The subchloroplast fractions 10 k and 144 k were isolated by differential centrifugation of digitonin disrupted plastids as in [17]; they were solubilized and electrophoresed as above. Chl was determined according to [18].



3. Results

After electrophoresis of SDS-solubilized primary thylakoids according to [4], apart from CPI and the free pigment zone, which are resolved [9] by the method in [1], additional bands can be detected (fig.1, table 1). A band running at a position higher than CPI is occasionally resolved and it represents a minor constituent; this band has the mobility of that reported for CPIa, the oligomeric form of CPI [4]. Another band, running between CPI and the free pigment is also resolved, which is unstable at longer



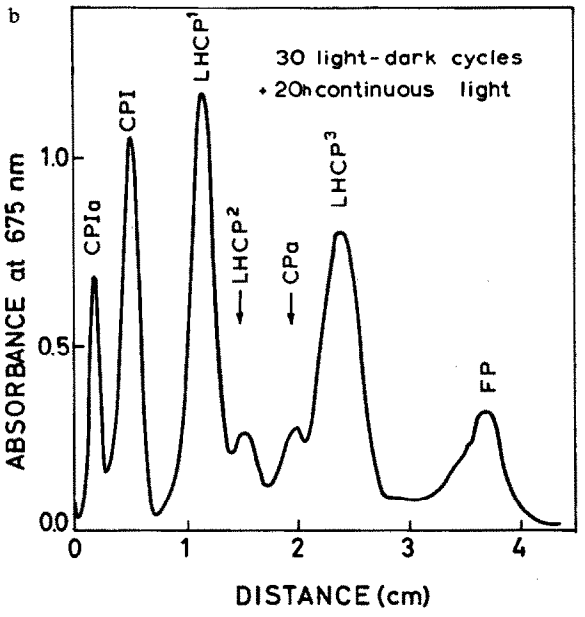


Fig.1. Distribution of chl between the chl–protein complexes resolved by SDS–gel electrophoresis according to [4] from SDS-solubilized thylakoids of *Phaseolus vulgaris* leaves. (a) Etiolated leaves exposed to 43 or 96 light-dark cycles; (b) transferred to continuous light for 20 h after exposure to 30 light–clark cycles.

Table 1
Distribution of chlorophyll between the chlorophyll–protein complexes in thylakoids of *Phaseolus vulgaris* isolated at various stages of development

Sample	chl *a* / chl *b*	% pigment in complexes									SDS chl	Migration (cm)
		CPIa	CPI	LHCP[1]	LHCP[2]	CPa	LHCP[3]	FP	CPIs	LHCPs		
28 LDC	–chl *b*	–	31	–	–	40	–	29	31	–	10	2.5
28 LDC	–chl *b*	–	24	–	–	10	–	66	24	–	50	2.0
40 LDC[a]	–chl *b*	–	37	–	–	42	–	20	37	–	6	2.0
43 LDC (short run)	–chl *b*	–	28	–	–	36	–	36	28	–	10	1.0
43 LDC (long run)	–chl *b*	4	31	–	–	19	–	46	34	–	10	4.0
57 LDC	26	–	32	–	–	18	–	34	32	–	10	3.0
96 LDC	6	–	37	16	–	24	12	10	37	28	10	1.2
30 LDC + 17 h CL[a]	3.4	5	22	21	7	7	18	13	27	56	6	4
31 LDC + 20 h CL	3.1	7	16	24	6	5	27	12	23	57	10	4
31 LDC + 44 h CL	2.5	6	15	31	7	3	21	14	21	59	10	4
46 LDC + 19 h CL	4.5	3	22	17	5	6	18	21	25	40	10	4
81 LDC + 23 h CL	4.6	5	22	13	3	10	24	20	27	40	10	4
70 h CL	3.0	4	18	24	10	?	30	14	22	64	5	3
70 h CL	3.0	3	16	10	7	?	42	22	19	59	10	4

SDS–PAGE electrophoresis according to [4]. Thylakoids of 6 day etiolated bean leaves exposed to periodic light–dark cycles (LDC) or continuous light (CL), except for ([a]) where 7 day etiolated leaves were used

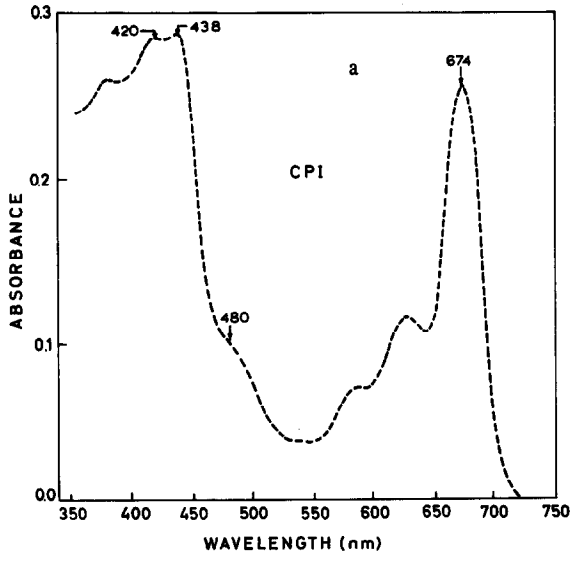
electrophoresis times (table 2) and higher SDS/chl ratio. Up to ~40% of the thylakoid chl can be resolved bound in this band, under conditions where the chl running as free pigment is reduced to 20%. The mobility and absorption spectrum of this complex (fig.2) suggest that it is identical to the one called CP*a* [4] or complex IV [3], and considered to be the reaction center of PS-II. In thylakoids of mature green leaves, the proportion of the thylakoid chl resolved bound on CPa is drastically reduced. Only CPIa, CPI, CPa and the free pigment band are resolved from thylakoids of leaves exposed to short periodic light treatment (≤ 40 light–dark cycles). However, after prolonged exposure to periodic light (vis 80 cycles) the light harvesting complexes can easily be detected (fig.1, table 1).

In leaves transferred to continuous light after short

Table 2
Distribution of pigment between the chl–protein complexes and the free pigment in thylakoids of intermittent light leaves, as affected by the duration of the electrophoretic run

Distribution of chl (%)			Migration (cm)
CPI	CPa	FP	
26	39	35	1.2
24	28	48	2.1
21	21	58	3.5
24	10	66	4.0

Thylakoids were isolated from 6 day etiolated bean leaves exposed to 41 light–dark cycles. Prior to electrophoresis they were solubilized in SDS in a SDS : chl ratio of 40 (w/w)



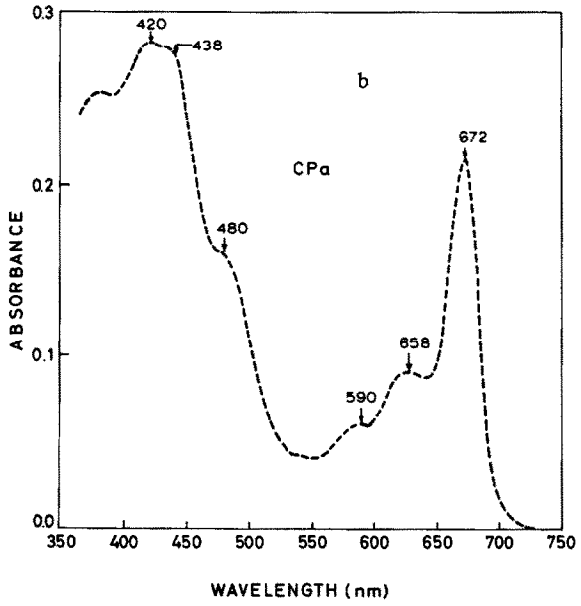


Fig.2. Absorption spectra of the chl–protein complexes separated by gel electrophoresis of SDS-solubilized primary thylakoids of etiolated leaves exposed to 43 light–dark cycles, in gel slices in situ. (a) CPI; (b) CPa. Spectra recorded in a Perkin Elmer 356 dual beam spectrophotometer.

pre-exposure to periodic light, all the light harvesting complexes are formed in excess and they represent the major amount of the thylakoid chl. This is also the case with the etiolated leaves transferred directly from darkness to continuous light; in a sample exposed to continuous light for 70 h, $\leqslant$ 60% of the thylakoid chl could be resolved bound on the 3 light harvesting complexes LHCP[1], LHCP[2] and LHCP[3]. For thylakoids formed after prolonged exposure to periodic light this figure is much lower (only ~25%). Prolonged pre-exposure to periodic light prior to transfer to continuous light, inhibits the formation of the light harvesting complexes, so that under these conditions the chl bound on these complexes is drastically reduced; this is in accordance with our earlier results [8,19].

Table 1 shows the effect of the SDS/chl ratio on the resolution of the complexes. The SDS/chl ratio affects on the one hand the dissociation of chl from the unstable complexes, resulting in varying amounts of the chl in the free pigment band, and on the other hand it affects the dissociation of the oligomeric forms of the complexes to the monomeric ones. In the primary thylakoids the SDS/chl ratio affects primarily the proportion of chl running as free pigment and especially the association of chl to the CPa polypeptide. In thylakoids of green leaves the SDS/chl ratio (and $\leqslant$ 15 that were tested) affects also the proportion of chl running as free pigment but it affects especially the distribution of chl between the 3 LHCPs. Lower ratios result in the formation of more LHCP[1], higher ratios of more LHCP[3]. In this case, even though the distribution of chl between the 3 LHCPs depends on the ability of SDS to dissociate the oligomeric forms to the monomer, the distribution of chl between the LHCPs (all of them together) and the rest of the complexes resolved is not greatly affected.

Since the light harvesting complexes are formed in excess under conditions where grana stacking is known to occur, and the thylakoids used in this study come from plastids with varying extents of grana stacking [8,9,12] we further analyzed SDS-solubilized thylakoid fractions (10 k and 144 k) obtained after digitonin disruption of mature green bean leaf chloroplasts, known to originate from grana or stroma lamellae [12,20]. The results are shown in table 3, fig.3. In this case also the light harvesting complexes represent the major component of the 10 k grana fraction. In addition, CPa seems to be present in a higher amount in this fraction. It is interesting to note that analysis of these fractions by the earlier method [1] gives similar results as far as the CPII : CPI ratio is concerned.

4. Discussion

The plastids formed after short exposure of etiolated leaves to periodic light are considered to contain small photosynthetic units: they saturate at higher light intensities than the mature chloroplasts, have higher light-saturated rates on a chl basis (PS-I, PS-II and CO_2 fixation) and much lower rate in their fluorescence induction kinetics [8,13,14]. Our findings that under such conditions most of the primary thylakoid chl *a* is bound on CPI and CPa strengthens the suggestion that these complexes originate from particles containing the reaction centers of PS-I and PS-II.

The light harvesting complexes are gradually

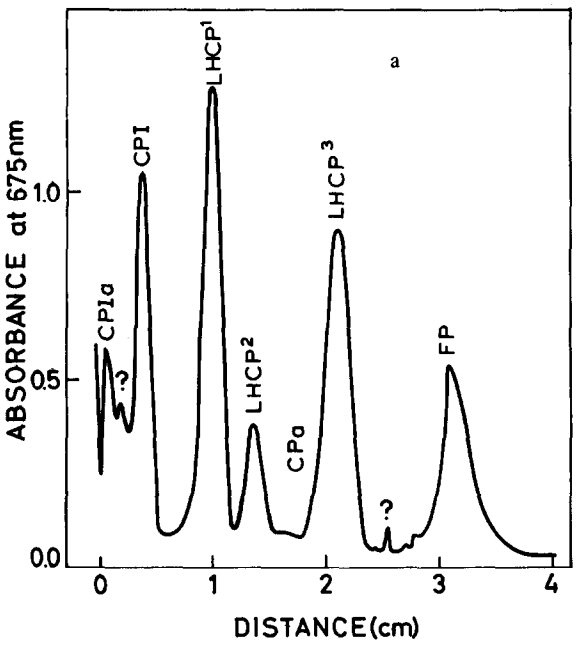
Table 3
Distribution of chl–protein complexes in thylakoids of chloroplasts and subchloroplast fractions of mature green leaves of *Phaseolus vulgaris*

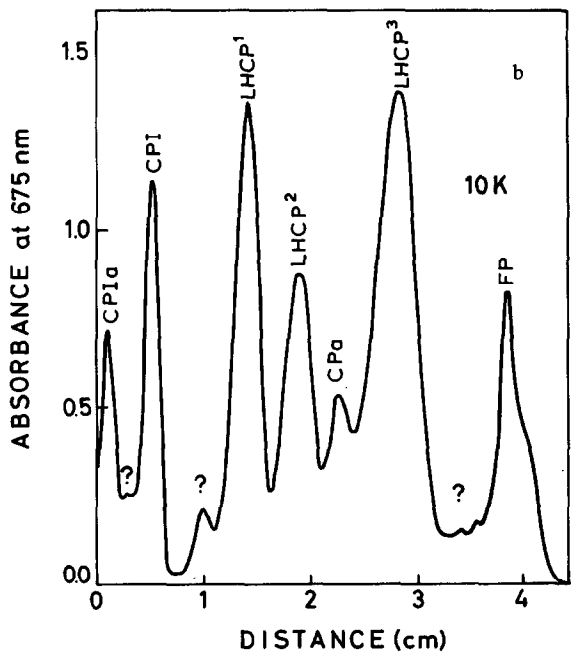
Sample	Distribution of pigment (%)									LHCPs
	CPIa	CPI	LHCP[1]	LHCP[2]	CPa	LHCP[3]	FP	CPIs	LHCPs	CPIs
I. Chloroplasts	9	16	25	6	1	23	16	25	54	2.16
10 k	6	10	17	13	4	32	15	16	62	3.87
144 k	9	29	12	7	3	21	15	38	40	1.05
		CPI				CPII	FP			
II. 10 k		10%				55	35			5.5
144 k		31				31	38			1.0

I. Analysis according to [4]; II. Analysis according to [1]

detected as exposure to periodic light is prolonged. This also agrees with the earlier finding that the half-rise time of the fluorescence induction kinetics in these plastids starts to decrease gradually [14], monitoring the gradual increase of the PS-II unit size.

Excessive amounts of light harvesting complexes are formed when the leaves are transferred to continuous light directly from darkness or after short exposure to periodic light. This occurs parallel to chl *b* and grana formation and, apart from the increase in the photosynthetic unit size, it also reflects the stacking of thylakoids in grana structures. This is also evident from the analyses of thylakoids of leaves transferred to continuous light after long pre-exposure to periodic light. Under such conditions the transformation of the primary thylakoid to the mature stage is drastically inhibited [8,19]. The parallel arrays of 'primary' thylakoids, formed after long exposure to





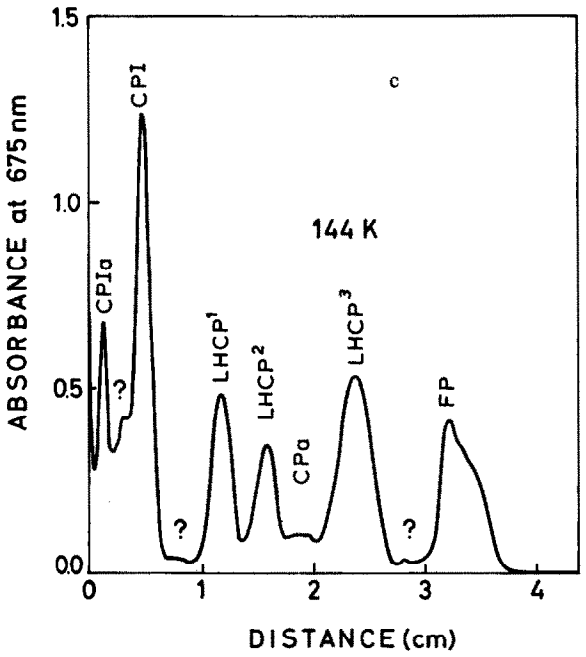


Fig.3. Distribution of chl between the chl–protein complexes resolved by SDS–gel electrophoresis according to [4] from SDS-solubilized thylakoids of chloroplasts and subchloroplast fractions obtained from 8 day etiolated bean leaves exposed to laboratory day–night conditions for 7 days: thylakoids before digitonin disruption of chloroplasts: (a) subchloroplast fractions isolated after differential centrifugation of digitonin disrupted chloroplasts; (b) 10 k; (c) 144 k.

periodic light, do not form grana stacks, in continuous light, to the extent that the short periodic light leaves or etiolated ones do, when transferred to continuous light [19]. Similarly they form reduced amounts of light harvesting complexes. Under these conditions only a small increase in the PS-II unit size can be determined [19] (the increase in the PS-I unit size remains unaffected [19]). This further suggests that for extensive growth of the PS-II unit size light harvesting complexes and grana formation should occur.

The formation of the light harvesting complexes and of chl *b* occurred in parallel, in accordance with [9,21,22]. This may show either that for the stability of the light harvesting complexes and their resolution, chl *b* is necessary [9,21,22], or that formation of chl *b* and of the protein of these complexes occur simultaneously. In any case, however, it seems that as soon as chl *b* starts being synthesized, the light harvesting complexes can be detected. This occurs after ~40–50 light–dark cycles in young etiolated leaves exposed to periodic light [15] or as soon as the lag phase in chl biosynthesis is over, in etiolated leaves exposed directly to continuous light [23,24].

The electrophoretic pattern and distribution of chlorophyll between the various complexes in the chl *b*-less primary thylakoid formed early in periodic light, is quite similar to that reported for the chl *b*-less mutant of barley [4]. Similarly, the pattern and distribution in thylakoids of mature green bean is similar to that of the wild-type barley [4]. On the other hand, the periodic light and continuous light plants resemble the barley mutant and wild-type, respectively, as far as photochemical activity is concerned [25]. This supports further the suggestion that thylakoids with small photosynthetic units and no chl *b* contain no light harvesting complexes, and have most of their chl *a* bound on CPI and CPa, the complexes considered to originate from particles containing the reaction centers of PS-I and PS-II.

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