CHLOROPHYLL–PROTEIN COMPLEXES OF HIGHER PLANT THYLAKOIDS: DISTRIBUTION, STOICHIOMETRY AND ORGANIZATION IN THE PHOTOSYNTHETIC UNIT

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1. Introduction

The photosynthetic pigments of higher plant chloroplasts are non-covalently bound to specific polypeptides; these pigment–protein complexes are the main intrinsic proteins of thylakoid membranes [1,2]. SDS solubilization of chloroplast thylakoids followed by SDS–polyacrylamide gel electrophoresis (PAGE) established the existence of two chlorophyll (chl)–protein complexes [3,4]: chl–protein complex 1 (CP1), the photochemically-inactive P700 chl *a*–protein of photosystem I (PSI), accounted for 10–18% of the total chl, the light-harvesting chl *a*/*b*–protein complex (LHCP) [5] accounted for 40–60% [1], and 25–50% consisted of free pigments complexed to SDS [1,2]. With improved techniques, other chl–protein complexes have been detected [6,11] and more chl remains associated with protein [12–16].

The distribution of chlorophyll between the pigment–protein complexes of higher plant thylakoids with different chlorophyll compositions has been examined using a SDS–PAGE procedure [14,18] which allows most of the chl to remain bound to protein. Despite the varying pigment compositions of the chloroplasts examined, the individual chl–protein bands from different species were electrophoretically and spectrally similar. The 6 chl–protein bands resolved are derived from 3 main complexes only: the PS I and PS II chl–protein complexes and the light-harvesting complex. Variations in the composition of the photosynthetic units of higher plants are due to the presence of different amounts of these 3 main complexes. The stoichiometry of these complexes in the photosynthetic unit of spinach thylakoids and a schematic model for their distribution is presented.

2. Methods

Spinach (*Spinacia oleracea* L.) plants were grown in water culture; pea (*Pisum sativum* L.), barley (*Hordeum vulgare* L.), *Sorghum bicolor* L. and *Panicum miliaceum* L. plants were grown in vermiculite. Leaves of the rainforest plants, *Alocasia macrorrhiza* (L.) G. Don. and *Helmholtzia glaberrima* (Hook.f.) Caruel. were obtained from a shaded gully. Chloroplasts were isolated from spinach, pea and barley [18] rainforest species [19] and mesophyll protoplasts and bundle sheath strands of *Panicum* and *Sorghum* [20] as described. Washed thylakoid membranes were resuspended in 50 mM Tricine buffer (pH 8.0) (2–4 mg chl/ml) and used immediately or stored in liquid N_2. [Chl] and chl *a*/chl *b* ratios were determined in 80% acetone [21]. SDS–PAGE [14] was performed at 4°C with minor modifications [18]. Chloroplast membranes were solubilized at 4°C, without prior lipid extraction, in 0.3 M Tris–HCl (pH 8.8), 10% glycerol, 0.5% SDS (final SDS/chl weight ratio of 10:1 and 0.5 mg chl/ml). Solubilized membranes (10–15 μg chl) were immediately applied to gels and the gels were run at 3 mA/gel at 4°C for 30–45 min. Absorption and fluorescence spectroscopy of gel slices and the relative distribution of chlorophyll are determined as in [14].

3. Results and discussion

When thylakoid membranes from 7 plant species were examined by discontinuous SDS–PAGE at 4°C, 7 chl-containing bands were resolved (fig.1). As shown with *Alocasia*, 6 of the bands contained protein, while the zone at the front consisted of chl and

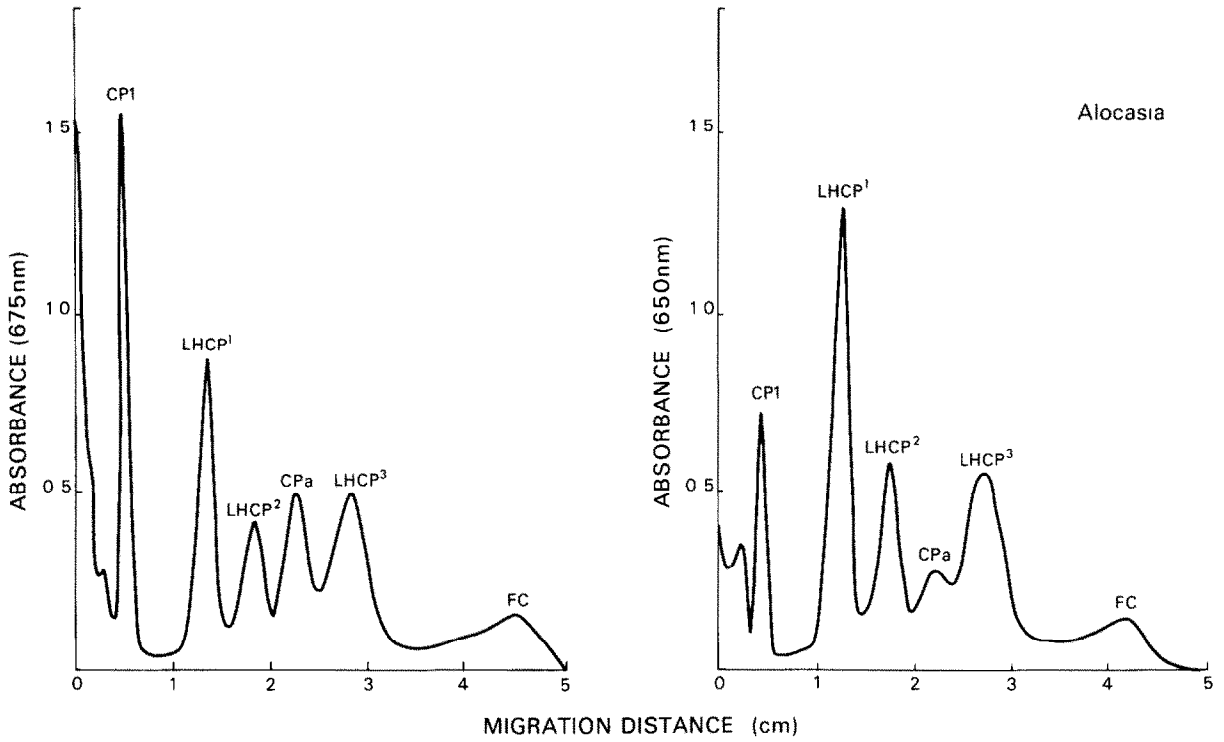

Fig.1. Gel scans at 675 and 650 nm of the chl–protein complexes resolved by SDS–PAGE according to [18] from SDS-solubilized *Alocasia* thylakoids.

carotenoids complexed with SDS. The 6 individual chl–protein bands had similar electrophoretic mobilities in each species examined. Their pigment compositions resolved were compared by absorption and fluorescence spectroscopy (not shown). In all cases, the pigment compositions of the individual chl–protein bands were similar. Hence, the chl–protein bands are similar to the spinach chl–protein bands characterized previously by absorption and fluorescence spectroscopy [14,18]. CP1a and CP1 are P700 chl *a*–protein complexes which together possess 1 P700 and 120 chl *a* molecules [18]. CPa, the third

Table 1
Relative distribution of chlorophyll in the chlorophyll–protein complexes of higher plant thylakoids

	% Total chlorophyll in complexes						
Thylakoids	CP1a	CP1	CPa	LHCP¹	LHCP²	LHCP³	FC
Spinach	21	9	10[a]	25	10	16	9
Pea	7	20	6	37	6	14	10
Barley	11	12	8	6	8	34	19
Helmholtzia	12	10	5	17	14	29	13
Alocasia	3	13	6	44	9	16	9
Panicum mesophyll	7	29	10	24	5	15	10
Panicum bundle sheath	7	21	8	20	10	22	12
Sorghum mesophyll	6	21	8	26	7	21	11
Sorghum bundle sheath	13	47	4	7	5	5	19

[a] Occasionally had 15% of the total in this band

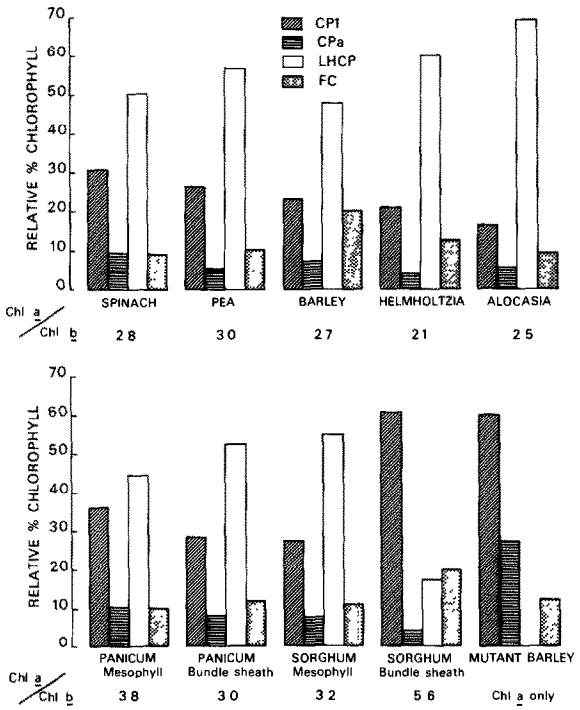

Fig.2. Relative distribution of chl between the chl–protein complexes resolved by SDS–PAGE [18] of some higher plant thylakoids. Chlorophyll contents of CP1a and CP1 were added together, and those of $LHCP^1$, $LHCP^2$ and $LHCP^3$ were combined, to give the total chl contents of the PSI chl–protein complex and LHCP, respectively.

chl *a*–protein complex has been inferred to be the PSII reaction centre complex [9,17]. This proposal has been strengthened since plastids with no LHCP, chl *b*-less mutant barley [22] or flashed-light plastids [23] have greater amounts of chl associated with CPa than usual. Further, the fluorescence properties of mutant barley CPa [22] are identical with those of a spinach digitonin PSII reaction centre complex [24]. With another SDS–PAGE method, CPa has been resolved into 2 chl *a*–protein bands [25,26]; genetic evidence with *Chlamydomonas* [25] and barley [26] indicates that these chl *a*–proteins belong to the PSII reaction centre complex. The 3 LHCPs have similar pigment, spectral and main polypeptide compositions [18]. Thus, the relative chl contents of CP1a and CP1 were added together, and those of the 3 LHCPs were also combined to give the total chl content of the PSI chl–protein complex and the light-harvesting complex, respectively. Comparison of the relative distribution of chl (table 1, fig.2) showed that different amounts of chl were associated with the 3 main complexes.

For spinach, pea and barley thylakoids, the PSI complex (CP1a and CP1) constituted 23–30% of the total chl *a*; less chl (6–10%) was associated with CPa and most was located in LHCP (fig.2). The distribution is similar to that reported for other sun plant species [13,14]. Shade plant chloroplasts have more chl *b* relative to chl *a* than is found in sun plant chloroplasts [2]. The distribution of chl in the pigment–protein complexes of two shade plant species, *Alocasia* and *Helmholtzia*, is significantly different from that found in sun plant species (fig.2). Both *Alocasia* and *Helmholtzia* have less chl associated with CP1a and CP1, while the CPa values are similar to those of the sun plant species. There was a concomitant increase in the amount of chl associated with the combined LHCPs, a maximum of 69% occurring in *Alocasia* LHCP (fig.2).

The dimorphic chloroplasts of C_4 plants have either similar or different pigment compositions [2]. *Panicum* is an NAD–malic enzyme C_4-plant in which the bundle sheath and mesophyll chloroplasts have similar pigment compositions (fig.2). In this case, the distributions of chl in the pigment–protein complexes were not greatly different (fig.2). On the other hand, with *Sorghum* (an NADP–malic enzyme species), the bundle sheath chloroplasts have decreased amounts of chl *b* relative to chl *a* compared to mesophyll chloroplasts. The amount of chl associated with the pigment–protein complexes in *Sorghum* mesophyll chloroplasts was rather similar to those of spinach and pea thylakoids (table 1). There was a marked difference with *Sorghum* bundle sheath chloroplasts, however, as the amount of chl associated with CP1a and CP1 was greatly increased to 60% of the total chl, while that with LHCP was only 17% (fig.2). Thus, *Sorghum* bundle sheath chloroplasts have much more chl associated with PSI and less with PSII than usual. This is consistent with the differences in photochemical activities of the photosystems of the bundle sheath chloroplasts of NADP–malic enzyme species, which have high levels of PSI and low levels of PSII [17,28].

Since all of the chl *b* is associated with LHCP, an inverse relationship exists between the chl *a*/chl *b* ratios of chloroplast thylakoids and their LHCP content (resolved by electrophoresis) as demonstrated by the earlier gel method [29] where only 1 LHCP was resolved. On the basis that the chl *a*/chl *b* ratio of

LHCP is 1.3–1.0 [1], the calculated chl *a*/chl *b* ratios of chloroplasts with varying amounts of LHCP (fig.2) are comparable to the actual chloroplast ratios, except for *Sorghum* bundle sheath chloroplasts.

The variations in the distribution of chl within the various complexes in the (PSU) of different chloroplasts is an important feature in their function and molecular structure. These results suggest that the chl of higher plant thylakoids is distributed between 3 main pigment–protein complexes: the PSI and PSII chl–protein complexes which contain P700 and P680, respectively, and the LHCP. The pigment molecules of each of these supramolecular complexes are probably bonded to several different polypeptides, but the individual chl–polypeptides are not unequivocally identified. Chloroplasts with higher chl *a*/chl *b* ratios and less LHCP, have more chl associated with the reaction centre complexes: e.g., *Sorghum* bundle sheath chloroplasts have 60% of the total chl in the PSI chl–protein complex. On the other hand, shade plant chloroplasts with low chl *a*/chl *b* ratios and higher amounts of LHCP, have lower amounts of chl in the reaction centre complexes: e.g., only 16% of the chl of *Alocasia* is associated with the PSI chl–protein complex. The chl *b*-less mutant barley with no LHCP, has twice as much chl associated with the PSI complex than with the PSII complex [22].

A model to account for the functional organization of the chlorophylls of the 3 main chl–protein complexes of the PSU of spinach thylakoids is shown in fig.3. The PSU of spinach thylakoids has 1 P700 and 1 P680 molecule for every 400 chl *a* and chl *b* molecules and an average chl *a*/chl *b* ratio of 2.7. The PSI chl–protein complex, which accounts for 30% of the total chl, has 1 P700/120 chl *a* molecules in agreement with [13,15,18], while the LHCP accounts for some 53% of the total chl. The PSII chl–protein complex resolved as CPa which is assumed to contain P680, has occasionally accounted for 15% of the total chl. It has been given 60 chl *a* molecules (table 1). Thus, the PSI chl–protein complex contains twice as much chl as that of PSII. Both PSI and PSII chl–protein complexes contain more chl *a* than suggested in earlier models [2,30].

While the stoichiometry of the chl distribution between the chl–protein complexes of spinach thylakoids is clear, actual models for their arrangement in the PSU are hypothetical [29]. Most models proposed are of the continuous array type [30–35] where the LHCP is arranged as a continuous array in

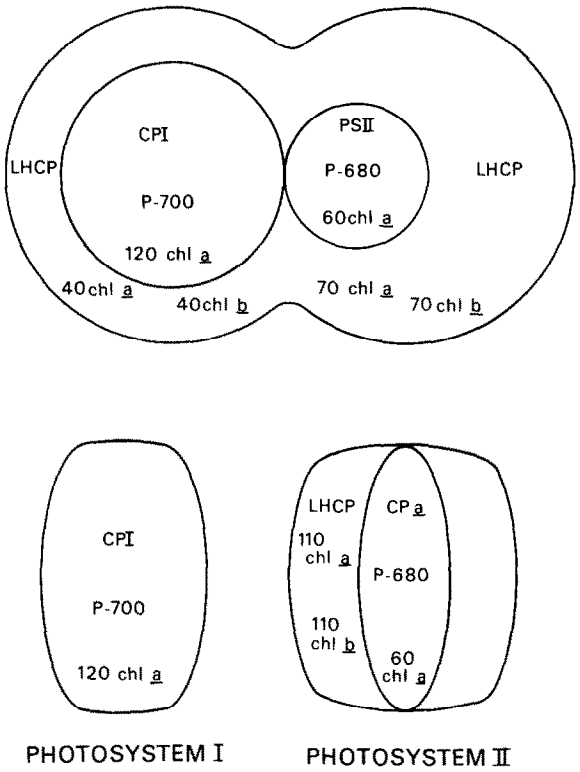

Fig.3. Schematic models of the chl–protein complexes in the photosynthetic unit of spinach thylakoids: (a) essentially separate package model where both PSII and PSI chl–protein complexes can interact to allow sharing of light energy; (b) extreme separate package model where LHCP is associated with PSII only. Most of the PSII complex and LHCP are located in the grana partitions, while the PSI complex is found in the non-appressed stroma thylakoids.

contact with both photosystems, although primarily with PSII. In contrast, an essentially separate package model was proposed [2] where each photosystem had its own complement of LHCP, but the photosystems could interact if necessary. This essentially separated package model [2] is revised (fig.3a) to allow for the relative chl contents of the PSI and PSII complexes which are now determined more accurately. However, our results indicate a lateral heterogeneity in the distribution of chl–protein complexes along spinach thylakoid membranes [36]. The PSI chl–protein complex is located mainly in the non-appressed membranes of stroma thylakoids and grana end membranes and margins. In contrast, the PSII chl–protein complex and the LHCP are located mainly in the appressed membranes of grana thylakoids. Moreover,

a rather constant ratio of the amount of PSII chl–protein complex to that of LHCP in both the partition region and the non-appressed membranes, suggests that LHCP is associated with PSII chl–protein complex rather than with PSI chl–protein complex. The compartmentation of the photosystems in spatially separated membrane regions suggests an even more separate package model for the distribution of most of the chlorophyll of grana-containing chloroplasts (fig.3b).

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