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Subpopulations of the main chlorophyll *a/b* light-harvesting complex of Photosystem II – isolation and biochemical characterization

Michael Spangfort [1,*] and Bertil Andersson [2]

[1] *Department of Biochemistry, University of Lund, Lund and* [2] *Department of Biochemistry, Arrhenius Laboratories, University of Stockholm, Stockholm (Sweden)*

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Spinach LHC II was fractionated into two distinct subpopulations by preparative isoelectric focusing. The method involves further solubilization of isolated LHC II by a mixture of Triton X-100 and octyl glucoside followed by free isoelectric focusing in a glass column. Two distinct green bands were resolved, one focusing at pH 4.45, comprising 42% of the chlorophyll, and one focusing at pH 4.30, comprising 58% of the chlorophyll. No band representing undissociated bulk LHC II could be seen. The alkaline fraction contained predominantly the 27 kDa polypeptide of LHC II, while the acidic fraction contained both the 27 and 25 kDa polypeptides. The two fractions therefore resemble the inner and outer pools of LHC II as suggested from subfractionation experiments on phosphorylated and heated thylakoid membranes. The two LHC II subpopulations show identical chlorophyll content and organization. The 27 kDa polypeptide of both fractions can be resolved into four species by denaturing isoelectric focusing. It was found that the relative abundance of the two most basic forms differs between the two LHC II subpopulations. The 25 kDa polypeptide is resolved into two molecular forms. Both subpopulations are, after isolation, predominantly (75%) present in their oligomeric form. The inner pool of LHC II is more heavily aggregated by Mg^{2+} ions as compared to the outer LHC II pool. The mechanistic significance of two LHC II populations with equal absorption properties but with different polypeptide composition will be discussed in terms of regulatory flexibility of light-harvesting.

Introduction

The light-harvesting apparatus of higher plant thylakoids consists of a number of different chlorophyll-protein complexes which are responsible for the absorption and transfer of excitation energy to the photosynthetic reaction centres. The chlorophyll binding protein complexes can be isolated as units, each containing a specific set of polypeptides and bound pigments [1,2]. Photosystem II contains both chlorophyll *a* and chlorophyll *a/b*-protein complexes. In close association with the reaction centre D_1/D_2-protein heterodimer [3] are the two chlorophyll *a* binding complexes; CP47 and CP43 [2]. There are also several minor chlorophyll *a/b* complexes; CP29, CP27 and CP24 [2,4]. However, the dominating constituent of the Photosystem II antenna is the light-harvesting chlorophyll *a/b* complex, designated LHC II. Up to 70% of the chlorophyll associated with Photosystem II is bound to LHC II which possesses a chlorophyll *a/b* ratio of about 1 : 1 [1,4].

The apopolypeptides of LHC II which are integral and membrane-spanning [5–8], are encoded by multiple genes in the nuclear DNA [9] and synthesized on cytoplasmic ribosomes as higher molecular weight precursors [10]. Depending on the plant species different numbers of LHC II apopolypeptides are seen [11]. In spinach thylakoids, the predominant subunits are a 27 kDa polypeptide and a 25 kDa polypeptide with the relative stoichiometric ratio of 4 : 1 [12]. Upon kinase-

* Present address: Max-Planck-Institut für Biophysik, Abt. Molekulare Membranbiologie, Frankfurt/Main, F.R.G.

Abbreviations: CHAPS, 3-((3-cholamidopropyl)dimethylammonio)-1-propane sulphonate; DAN, diazoacetyl norleucine methyl ester; PCMB, *p*-chloromercuribenzoate; PMSF, phenylmethylsulphonyl fluoride; SDS, sodium dodecyl sulphate; Tricine, *N*-(tris-(hydroxymethyl)methylglycine.

Correspondence: B. Andersson, Department of Biochemistry, Arrhenius Laboratories, University of Stockholm, S-106 91 Stockholm, Sweden.

mediated protein phosphorylation of thylakoid membranes, the apopolypeptides of LHC II become phosphorylated [13]. In spinach the 25 kDa polypeptide shows both a more rapid phosphorylation kinetic and a higher degree of specific phosphate incorporation as compared to the 27 kDa polypeptide [12,14,15].

Early electrophoretic analysis by Green and Camm [16] resolved LHC II into two closely migrating chlorophyll-containing bands of different polypeptide composition. This suggested that LHC II in itself is heterogeneous. More recent subfractionation studies of spinach thylakoid membranes have suggested that LHC II consists of two subpopulations which differ in their relative proportion of the 27 kDa and the 25 kDa polypeptide and in their association with Photosystem II [14]. One subpopulation, tightly bound to the core of Photosystem II, is entirely dominated by the 27 kDa polypeptide. The other LHC II subpopulation, more peripherally located with respect to Photosystem II has a relatively high abundance of the 25 kDa polypeptide. This peripheral or outer LHC II pool can reversibly dissociated from Photosystem II in response to protein phosphorylation or moderate heat and it has therefore been suggested to be responsible for the short term acclimation of the Photosystem II light-harvesting apparatus [14,17]. Studies on LHC II isolated from leaves grown at different light intensities show that the outer pool is also responsible for the long-term acclimation of the Photosystem II antenna [18,19].

In a previous communication, we reported that the LHC II preparation isolated after Triton X-100 treatment [20] contains both LHC II subpopulations and that these can be separated by isoelectric focusing under non-denaturing conditions [21]. In this study we present an improved isolated procedure for the two LCH II subpopulations including a characterization of their polypeptide composition, pigment organization, aggregation behaviour and oligomeric arrangement.

Materials and Methods

Urea, Amberlite MB-1 and Ampholines (no. 1809-111, 1818-106, 1818-126, 1818-116) were obtained from LKB Products, Sweden. CHAPS (No. 810) was from Boehringer-Mannheim, F.R.G. *N*-Octyl β-D-glucopyranoside (No. 0-8001) and Bicinchoninic Acid Protein determination kit were purchased from Sigma, U.S.A.

Isolation of thylakoid membranes

Thylakoid membranes were isolated from spinach leaves as in [20]. Protease inhibitors, when used, were 2 mM PSMF 1 mM PCMB/5 mM DAN + 5 mM $Cu(Ac)_2$/1,10-phenanthroline. Stock solutions were made up and added to the grinding medium as described in Ref. 22.

Isolation of bulk LHC II

Isolation of the bulk LHC II was performed according to Ref. 20 with modifications described in Ref. 23. Isolated thylakoid membranes were solubilized with Triton X-100 and LHC II was isolated by sucrose gradient centrifugation, followed by 300 mM KCl induced aggregation [24] of the purified LHC II.

Isolation of LHC II subpopulations

The LHC II subpopulations were isolated from purified bulk LHC II by non-denaturing isoelectric focusing. The LHC II was suspended in destilled water and collected by centrifugation 40 000 × *g* for 30 min. The pellet was solubilization by the addition of a solution containing 1% Triton X-100, 0.5% *n*-octyl β-D-glucopyranoside, 0.5% glycine, 1.5% ampholine (pH 5.0–3.5), 0.5% ampholine (pH 4.5–2.5). The final concentration of LHC II was 1 mg chlorophyll/ml. After stirring for 5 min on ice, sucrose was added to a final concentration of 54.0% and the sample was loaded on to a LKB 8100 ampholine electrofocusing column. The sample was carefully overlayed with a 50–5% sucrose gradient containing 0.5% Triton X-100, 0.3% *n*-octyl β-D-glucopyranoside, 0.5% glycine, 1.5% ampholine (pH 5.0–3.5) and 0.5% ampholine (pH 4.5–2.5). The electrofocusing was run in the dark for 72 h at 5 W constant power. The anode solution was 0.25 M H_3PO_4 and the cathode solution was 0.25 M NaOH in 60% sucrose. Stock solutions of Triton X-100 and *n*-octyl β-D-glucopyranoside were stored in Amberlite MB-1.

The chlorophyll-containing fractions obtained by the isoelectric focusing were collected and dialysed against 0.2 M NaCl/20 mM Tricine (pH 7.5) for 2 × 1 h and against 5 mM NaCl/5 mM Tricine (pH 7.5) overnight in the dark. The samples were concentrated by centrifugation using Centricon concentrators with a 30 kDa cut-off filter. Alternatively, the samples were dialysed as described above and treated with Bio-Bead for 2 h. KCl was then added to a final concentration of 300 mM (pH 7.2). After incubation at room temperature for 5 min with stirring, the samples were collected as a pellet by centrifugation at 40 000 × *g* for 15 min. Unless otherwise stated all preparative steps were performed at 4° C.

Chlorophyll and protein determination

Chlorophyll was determined in 80% acetone according to Arnon [25]. Protein was determined spectroscopically at 562 nm using the bicinochoninic acid method described in Ref. 26.

SDS-polyacrylamide gel electrophoresis and analytical isoelectric focusing

The oligomeric and monomeric form of the isolated LHC II were resolved by mild polyacrylamide gel electrophoresis [27] using a SDS/chlorophyll ratio of 7.5 for solubilization. The gels were scanned at 663 nm to

quantify the resolved chlorophyll containing bands. Denaturating SDS-polyacrylamide gel electrophoresis was performed according to Laemmli [28] at 25°C using a 12–23% or a 8–15% polyacrylamide gradient.

Denaturing isoelectric focusing was performed mainly according to [29] using 3 × 180 mm glas tubes. Samples were delipidated at room temperature by extraction with 80% acetone containing 5% mercaptoethanol followed by centrifugation at 14000 × *g* for 10 min. The colourless protein pellet was solubilized by stepwise addition of urea and detergents (SDS, CHAPS) at initially high pH. For this purpose the solutions A, B, C of Ref. 29 were used with the following modifications. pH in solution A was raised from pH 7.5 to pH 8.5. The ampholine content in solution B was 2.7% ampholine (pH 8.0–5.0), 0.7% ampholine pH (6.5–4.0). The ampholine content in solution C was 0.6% ampholine (pH 8.0–5.0), 0.15% ampholine pH (6.5–4.0). The tube gels contained 4.5% acrylamide, 2% CHAPS, 9.15 M urea, 1.2% ampholine (pH 8.0–5.0) and 0.3% ampholine (pH 6.5–4.0). Stock solution of CHAPS was stored in Amberlite MB-1. The tube gels were run at 50 V for 30 min, 200 V for 30 min and 400 V for 18 h at 12°C.

For two-dimensional isoelectric focusing/SDS-polyacrylamide gel analysis the isoelectric focusing tube gels were equilibrated 3 × 10 min in the solubilizing buffer of [28] under gentle agitation, loaded on to an 8–15% SDS-polyacrylamide gel and adhered with 1% agarose in 0.1% SDS/10% glycerol/62.5 mM Tris-HCl (pH 6.8). The second dimension SDS-polyacrylamide gels were run using the gel system of Laemmli [28].

All gels were stained with Coomassie brilliant blue R-250, destained and scanned by a LKB 2202 laser densitometer.

Aggregation measurements

LHC II samples, concentrated by centrifugation and filtration, were suspended in 50 mM sucrose/2 mM Tricine (pH 7.5) to a final concentration of 50 μg chlorophyll/ml. Changes in 180° light scattering induced by 0–10 mM $MgCl_2$ was monitored as in Ref. 23 by measuring absorbance changes at 550 nm using a Shimadzu UV-3000 spectrophotometer.

Spectrophotometric analysis

Absorption difference and derivative spectra, were recorded on a Shimadzu doublebeam UV-3000 spectrophotometer at room temperature. Samples were suspended in 50 mM sucrose/2 mM Tricine (pH 7.5) to approx. 15 μg chlorophyll/ml. Difference spectra were normalized at the absorption peak of the samples. Derivative spectra were performed with $\Delta\lambda = 0.9$ nm.

Low-temperature (77 K) fluorescence emission and excitation spectra were measured as described in Ref. 30.

Results

The existence of at least two LHC II subpopulations with different polypeptide composition, as suggested from protein phosphorylation and subfractionation studies [14,31,32] and from electrophoretic analysis [16], was strengthen by subfractionation of purified bulk LHC II by isoelectric focusing [21]. Three fractions were obtained. One, focusing at pH 4.45, contained only the 27 kDa polypeptide while another, focusing at pH 4.30, was significantly enriched in the 25 kDa polypeptide. These two fractions resembled the inner and outer subpopulation of LHC II respectively. Apart from these two fractions a third major chlorophyll containing band was obtained containing about half of the focused chlorophyll. This fraction was resolved at pH 4.38 and contained undissociated bulk LHC II. The presence of this material prevented our making a complete subfractional analysis of LHC II.

In order to improve the solubilization of LHC II, different types and amounts of detergents were used. Moreover, the composition of the medium during the isoelectric focusing was varied. None of these approaches was successful. The modification that solved the problem concerned the aggregation of the LHC II preparation prior to solubilization for isoelectric focusing. The normal precipitation of LHC II by 5 mM Mg^{2+} during isolation [23] was changed for precipitation by 300 mM KCl. When such precipitated material was solubilized in 1% Triton X-100 and 0.5% *n*-octyl β-D-glucopyranoside and fractionated by preparative isoelectric focusing only two bands, focusing at pH 4.30 and 4.45 respectively, were obtained (Fig. 1). The intermediate fraction, containing the undissociated LHC II

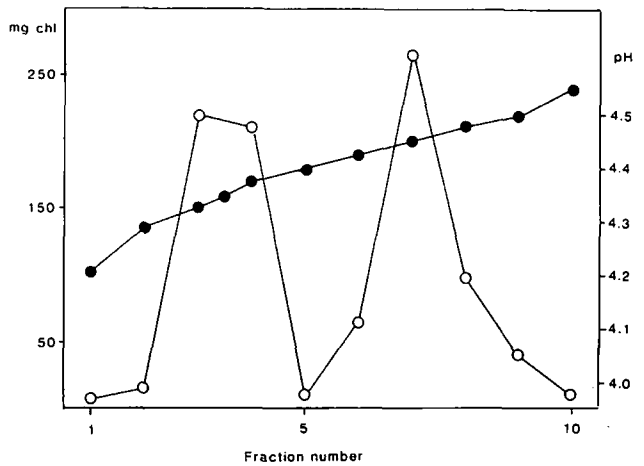

Fig. 1. Chlorophyll distribution after isoelectric focusing of bulk LHC II (○). The pH of collected fractions (●) has been indicated. The left-hand peak represents LHC II with a high relative content of the 25 kDa polypeptide (outer LHC II), while the right-hand peak represents material entirely dominated by the 27 kDa polypeptide (inner LHC II).

material, was no longer present and there was a concomitant increase of chlorophyll in the bands focusing at pH 4.30 and 4.45.

SDS-polyacrylamide gel electrophoresis revealed that the acidic band was relatively enriched in the 25 kDa polypeptide having a 27/25 kDa polypeptide ratio of 1.5–2.5 while the alkaline fraction consisted of the 27 kDa polypeptide only. Since no undissociated LHC II was found our present observation clearly shows that all of the LHC II is composed of two main subpopulations where the acid and basic fractions correspond to the outer and inner LHC II, respectively. The broadness of the acidic fraction may indicate some further heterogeneity of the outer LHC II subpopulation. The addition of proteinase inhibitors in the grinding medium during the isolation of bulk LHC II did not alter the banding pattern after isoelectric focusing nor the relative distribution of chlorophyll between the two fractions.

The distribution of chlorophyll between the two fractions was uneven. The acidic fraction contained 58% of the chlorophyll, whereas the alkaline fraction contained 42%. This chlorophyll proportion between the two subpopulations will probably vary in leaf tissue grown at different light intensity, since the size of the outer LHC II pool increases at low light conditions while the size of the inner remains constant [18,19].

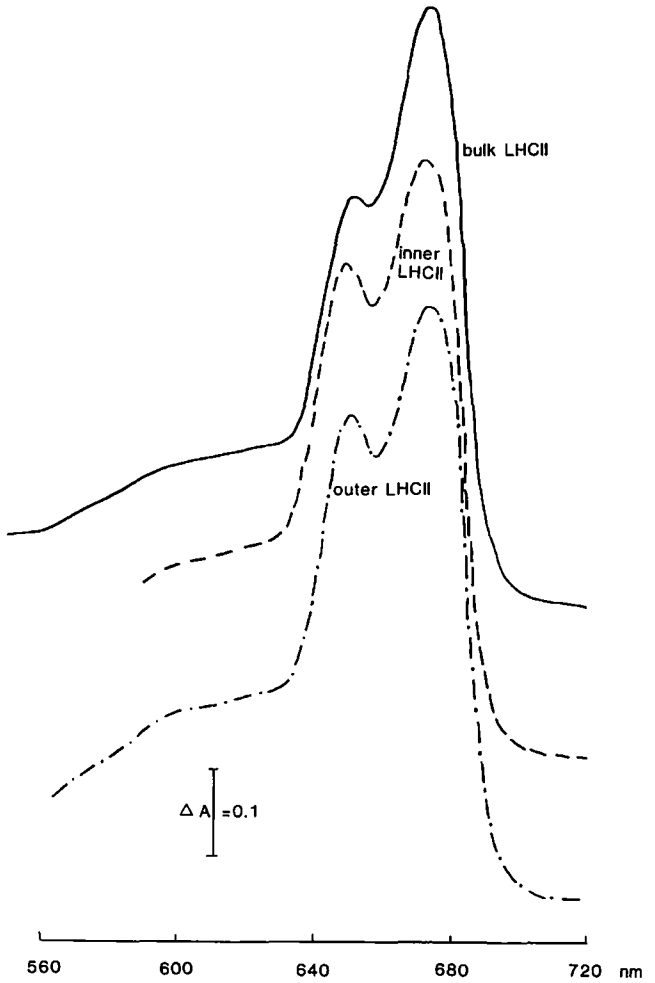

Fig. 2. Absorption spectra of the bulk LHC II and the isolated inner and outer LHC II subpopulations.

Pigment analysis

The chlorophyll to protein ratio (mol : mol) of the two fractions was 8 chlorophylls/polypeptide, whereas the bulk LHC II contained 10 chlorophylls/polypeptide. Thus, there is some loss of chlorophyll during the further solubilization and isoelectric focusing of the bulk LHC II. The overall stoichiometric relation between chlorophyll and protein is within the range of reported values 7–13 for LHC II [20,33].

The absorption spectra of the two LHC II subpopulations and the bulk LHC II in the 550–720 nm range are shown in Fig. 2. The bulk LHC II shows two main absorption peaks at 675 nm and 652 nm, due to chlorophyll *a* and chlorophyll *b*, respectively. The isolated subpopulations both show the same principal absorption peaks, but these are blue-shifted 1–2 nm. The difference spectrum between the two LHC II subpopulations did not reveal any differences and their derivative spectra obtained from absorption scanning were almost identical. The relative amplitude for the chlorophyll *b* peak at 650 nm is the same for both subpopulations. In addition, direct measurements of the chlorophyll *a/b* ratio [25] revealed that both LHC II subpopulations have equal amounts of chlorophyll *a* and chlorophyll *b* (chlorophyll $a/b = 1.0$). The bulk LHC II showed a ratio of 1.2. This decrease in the relative chlorophyll *a* content is probably due to removal of contaminating chlorophyll-*a*-rich chlorophyll proteins during the isoelectric focusing and/or loss of chlorophyll during the isolation. We have found that CP29, possessing a chlorophyll *a/b* ratio of 3.0 [2], is present in the bulk LHC II preparation. The contaminating CP29 was recovered at pH 4.65 and as a result does not contaminate the two subfractions. This removal of CP29 explains, at least in part, the small blue-shift in the spectra (Fig. 2) of the two LHC II subpopulations.

Low-temperature (77 K) fluorescence emission spectra of the two LHC II subpopulations were recorded. Both fractions showed an identical spectrum with an emission peak at 679 nm. Moreover, the 77 K fluorescence excitation spectra in the range of 400–500 nm measured at the emission peak wavelength were the same with broad peaks at 440 nm and 470 nm.

In conclusion the two LHC II subpopulation appear to be very similar with respect to chlorophyll content and organization. Moreover, the isolation by isoelectric focusing does not seriously disturb the functional integrity of the two subpopulations.

Polypeptide analysis

In contrast to the pigment composition the two LHC II subpopulations differ in their polypeptide composition. In view of reported polypeptide complexity [11,32] and gene heterogeneity [9] of LHC II, the polypeptide composition of the isolated subpopulations were analyzed in more detail. In particular it was of interest to see whether the 27 kDa species of outer and inner LHC II were the same. Fig. 3 (top gels) shows tube gel isoelectric focusing pattern of the two LHC II fractions under denaturing conditions. The inner LHC II, apparently containing one single 27 kDa polypeptide, as judged by SDS-polyacrylamide gel electrophoresis, could be resolved into three major bands and one minor band, all focusing in the range of pH 4.40–4.70. When the outer LHC II subpopulation, containing both the 27 and 25 kDa polypeptide was analyzed, two additional bands appeared in the same pH range. These focused very close to the isoforms of the 27 kDa polypeptide and an accurate analysis could not be done (Fig. 3, top gel, right). Therefore, two-dimensional electrophoresis was performed using isoelectric focusing in the first dimension and SDS-polyacrylamide gel electrophoresis in the second dimension (Fig. 3). This approach gave a well-resolved polypeptide pattern of both LHC II subpopulations. As suggested from the first-dimension gel the inner LHC II subpopulation was resolved into four 27 kDa polypeptides (designated 27 a–d). Their relative abundance was determined by laser densitometry (Table I). The two most basic isoforms (27 c and 27d) are the most prominent, comprising 36% and 32% of the total 27 kDa polypeptide mass, respectively. The abundance of the most acidic form (27a) is quite low (9%). Interestingly, the outer LHC II population also gave rise to four 27 kDa species. Each of these focused at an identical pH to a corresponding 27 species from the inner LHC II subpopulation (Fig. 3). In addition, the relative abundance of the 27a and 27b species is about the same in both subpopulations (Table I). However, the relative amount of the 27c and 27d components is different. In the outer LHC II pool the 27c/27d polypeptide ratio is 1.8, while it close to unity in the inner pool.

TABLE I

Relative distribution (%) of the 27 kDa and 25 kDa polypeptide molecular species of the inner and outer LHC II subpopulations after two-dimensional gel electrophoresis

The percentage of the 27 kDa and 25 kDa polypeptide subspecies within each subpopulation were determined by laser densitometer scanning of SDS-polyacrylamide gels after two-dimensional electrophoresis. The a–d polypeptide subspecies refer to those indicated in Fig. 3. The relative amounts are mean values of the peak areas obtained by scanning the SDS-polyacrylamide gels in the *x*-axis and *y*-axis. The total 27/25 kDa ratio of the outer LHC II pool was 2.3, while that of the inner LHC II pool was above 10.

Subspecies	27 kDa polypeptide		25 kDa polypeptide	
	inner LHC II	outer LHC II	inner LHC II	outer LHC II
a	9	10	(44)	43
b	23	25	(56)	57
c	36	42		
d	32	23		

As revealed in Fig. 3, the 25 kDa polypeptide of the outer pool was resolved into two bands (designated 25a and 25b). The 25a and 25b polypeptide focused at pH values very similar to the 27b and 27c polypeptides, respectively. The 25b polypeptide is somewhat more abundant than the 25a polypeptide (Table I). The inner LHC II contains small amounts of the two 25 kDa polypeptides in the same proportion as in the outer LHC II pool. They are the result of contamination from

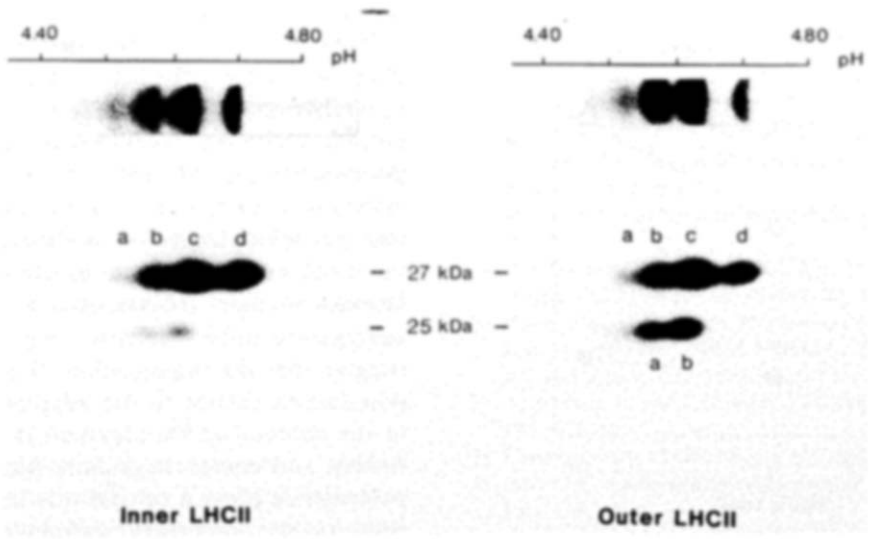

Fig. 3. Polypeptide composition of inner and outer LHC II subpopulations separated under denaturing conditions by isoelectric focusing (inset) followed by SDS-polyacrylamide gel electrophoresis.

the outer LHC II pool during collection of the samples from the isoelectric focusing column. The complex pattern obtained after isoelectric focusing is in agreement with a recent study of Bassi et al. [32]. It was shown that the more acidic LHC II components were the most heavily phosphorylated, and this confined these to the outer or mobile pool of LHC II.

Oligomeric arrangement and aggregation behaviour

Electron microscopy of two-dimensional crystals and image analysis suggest that the LHC II is arranged in trimeric units in the thylakoid membrane [34]. When analysed by mild SDS-PAGE, LHC II often give rise to multiple bands, since the electrophoretic migration of oligomeric forms is slower than the monomeric form. Using mild SDS-PAGE [27] the relative proportion of the monomeric form ($LHCP^3$) and oligomeric (most likely trimeric) forms ($LHCP^1$) of the two LHC II subpopulations were analyzed. It was found for both subpopulations that 75% of the material was recovered in the oligomeric form and about 20% in the monomeric form. Approx. 5% was free chlorophyll. Thus both LHC II subpopulations are in the same oligomeric state in their isolated form. It can therefore be concluded that the formation of oligomers is not dependent on the relative amount of the 27 and 25 kDa polypeptide.

When isolated LHC II is incubated with Mg^{2+} ions it aggregates and forms membranous structures resembling in vivo grana stacks [35]. In order to compare the aggregation behaviour of the two isolated LHC II subpopulations these were incubated with $MgCl_2$ and analyzed for changes in their light-scattering behaviour (Fig. 4). The inner LHC II pool showed a very prompt aggregation in response to the added Mg^{2+}. In contrast, the response of the outer LHC II pool was not so pronounced. At 10 mM Mg^{2+} the relative scattering of the inner LHC II pool was 4-times higher than the outer

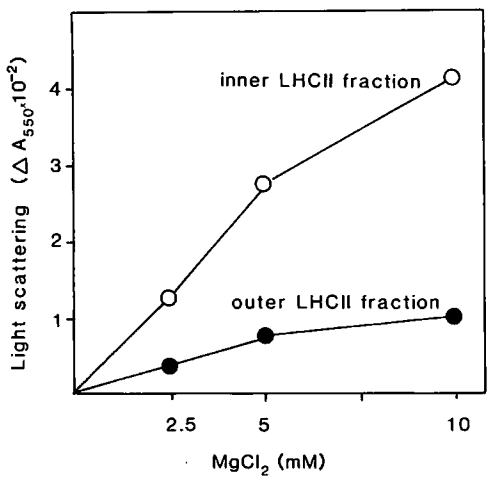

Fig. 4. Light-scattering changes (λA_{550} nm) induced by $MgCl_2$ when added to samples containing the inner or outer LHC II subpopulation. Samples contained 50 μg chlorophyll/ml.

pool. Even at lower concentrations, the scattering difference was quite obvious (Fig. 4). The presence of the 25 kDa polypeptide therefore seems to diminish the Mg^{2+}-induced aggregation, which may be due to a higher degree of exposed negative charges. The more acidic isoelectric point of the outer LHC II could be taken in support for such an explanation. At present, the physiological significance of this difference in aggregation behaviour between the inner and outer subpopulations of LHC II is hard to envisage.

Discussion

We could not find any major differences between the isolated LHC II subpopulations and the bulk LHC II with respect to pigment interaction and content and the ability to form oligomeric complexes. In contrast, the polypeptide composition of the two subpopulations is different. In particular, the 25 kDa polypeptide is confined to the outer LHC II pool. The two pools contain the same 27 kDa polypeptide isomers, but the relative content of the two most basic species (27c and 27d) differs. Despite this different polypeptide composition, the pigment of the two subpopulations is the same. This argues strongly that the pigment-binding properties of the 27 kDa polypeptide and 25 kDa polypeptide, including their isoelectric isomers, are the same.

Native LHC II have been reported to focus between pH 4.0 and pH 4.55 after non-denaturing isoelectric focusing [36–38]. This is in accordance with the present study. Under denaturing conditions however, the LHC II polypeptides are generally found to focus at a higher pH range, between 4.5–6.3 [29,39,40]. This was also the case for the constituent polypeptides of the inner and outer LHC II subpopulations. This discrepancy is probably due to removal of lipids and pigments from the LHC II and the inclusion of high amounts of urea for the denaturing isoelectric focusing analysis.

Even though the different polypeptide composition does not affect light-harvesting, it gives rise to some other distinct properties between the two LHC II populations. These are: (i) different degree of protein phosphorylation [12,15]; (ii) different ability to undergo reversible dissociation from Photosystem II due to protein phosphorylation or moderate heat [14]; (iii) only the outer pool changes in size in response to long-term changes in light conditions; (iv) the Mg^{2+}-dependent aggregation differs in vitro (Fig. 4). These differences suggest that the organization of LHC II into two subpopulations relates to the adaptation and organization of the antenna of Photosystem II rather than light-harvesting and energy migration. Most likely, the 25 kDa polypeptide plays a central role in this regulatory function. Under long-term acclimation to low light, the change in the antenna size of LHC II is due to a specific increase of the outer pool [18,19]. This means that there

is an increase in LHC II with a relatively high content of the 25 kDa polypeptide. Compared to the 27 kDa polypeptide the 25 kDa polypeptide is a very good substrate for the kinase which mediates the protein phosphorylation [12]. As a consequence of this phosphorylation, the outer LHC II population dissociates from the Photosystem II core and the inner LHC II and migrates to the stroma-exposed thylakoid regions. The presence of the 25 kDa phosphoprotein specifically in the portion of LHC II that increase its size under low light conditions allows for maximal flexibility and connects short-term and long-term acclimation of the antenna. In response to a sudden increase in light intensity after a period of limiting light the excess portion of the antenna can rapidly be disconnected from Photosystem II through the kinase-mediated phosphorylation of the outer LHC II. Such a specific regulatory system would not be possible if LHC II had a homogeneous polypeptide composition, i.e., only 27 kDa species. The presence of a 25 kDa polypeptide homologous to and with the same light-harvesting properties as the major 27 kDa polypeptide, but with distinct regulatory properties, probably provides one explanation for the heterogeneous gene family of LHC II.

Are the 27 kDa and 25 kDa polypeptides and their isoforms different gene products or post-translational modifications of the same gene products? The LHC II genes have been devided into two types, Type I and Type II, based on sequence comparison and the presence of an intron in the Type II gene (for review see Ref. 41). Although no differences in function have been attributed to the Type I and Type II gene products, this gene heterogeneity is preserved through evolution [42], which indicates its physiological significance. A possibility exists that the 27 kDa and the 25 kDa polypeptides reflect the gene products of Type I and Type II genes, respectively. This suggestion is supported by Pichersky et al. [43] who showed that the mature product of an in vitro translated LHC II Type I gene has about the same electrophoretic mobility as a 27 kDa LHC II polypeptide, while the same experiment using a Type II gene resulted in a mature product migrating close to a 25 kDa LHC II polypeptide. Whether the isoforms of the 27 and 25 kDa polypeptides are different gene products within the two gene families or are the result of posttranslational modifications like phosphorylation [13] or palmitoylation [44] can not be judged at present.

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