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Characterization of two different subpopulations of spinach light-harvesting chlorophyll *a*/*b*-protein complex (LHC II): polypeptide composition, phosphorylation pattern and association with Photosystem II

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The properties of the light-harvesting chlorophyll *a*/*b*-protein complex of Photosystem II (LHC II) was analysed in subfractions isolated from phosphorylated or heated thylakoids. The results showed that LHC II is heterogenous with respect to polypeptide composition, phosphorylation kinetics and structural association with the core of Photosystem II. One LHC II subpopulation is tightly bound to the core of Photosystem II and contains mainly a slowly phosphorylated 27 kDa polypeptide. The other subpopulation of LHC II is peripherally bound to the core and contains a relatively high proportion of a rapidly phosphorylated 25 kDa polypeptide. The latter pool of LHC II can reversibly detach from Photosystem II in respons to phosphorylation or elevated temperatures. These findings will be accommodated into a model for the dynamic arrangement of the antenna of Photosystem II.

Introduction

The light-harvesting assembly of Photosystem II in higher plants is composed of several different chlorophyll-proteins. Apart from two chlorophyll *a*-proteins (CP 47 and CP 43) there are reports on a number of chlorophyll *a*/*b*-proteins associated with Photosystem II [1–4]. The most abundant of these chlorophyll *a*/*b*-proteins is LHC II [1]. In addition to light-harvesting, LHC II is thought to mediate thylakoid membrane stacking [5,6]. Phosphorylation of LHC II is brought about by a membrane-bound kinase. Upon phosphorylation a fraction of LHC II separates from the Photosystem II core in the appressed membranes and migrates into the nonappressed thylakoid regions [7,8], thereby allowing for an increased association of LHC II with Photosystem I [9,10]. Lateral rearrangement of the LHC II occurs also at elevated temperatures [11,12]. In contrast to phosphorylation, heat induces Photosystem II with a portion of its LHC II to migrate into the stroma thylakoids leaving mainly free LHC II behind in the appressed membranes.

An intriguing feature of LHC II is a prominent heterogeneity at both protein and gene levels. The complex is composed of several distinct but closely

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Abbreviations: LHC II, light-harvesting chlorophyll *a*/*b*-protein complex of Photosystem II; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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homologous polypeptides in the 25–28 kDa range [13,14]. These polypeptides are specified by multiple nuclear genes [15] and synthesized in the cytoplasma as 4–5 kDa larger precursor proteins [16–18]. When analyzed by mild non-denaturating SDS-polyacrylamide gel electrophoresis ('green gels') the LHC II gives rise to three or more bands [1]. These are thought to represent different oligomeric forms of LHC II.

In spinach the predominant subunits of LHC II are a 27 kDa and a 25 kDa polypeptide. We have previously demonstrated that the two polypeptides showed different degrees of phosphorylation and lateral mobility [19]. The specific incorporation of phosphate into the 25 kDa polypeptide exceeded that of the 27 kDa polypeptide by a factor of three. Moreover, the LHC II subpopulation that migrated to the stroma thylakoids upon phosphorylation had a relatively high content of the 25 kDa polypeptide. In addition, it has been suggested that the rapid phosphorylation of the low-molecular-weight LHC II polypeptide is of functional importance in the regulation of excitation energy between the two photosystems [20].

In this study we have in detail characterized the heterogenous and dynamic organization of the light-harvesting apparatus of Photosystem II with respect to polypeptide composition, phosphorylation kinetics and membrane location.

Materials and Methods

Thylakoids were isolated [21] from spinach leaves (*Spinacia oleracea* L.). For phosphorylation, thylakoids were suspended in 15 mM Tricine (pH 7.8), 20 mM NaCl, 5 or 10 mM $MgCl_2$, 10 mM NaF, 100 mM sucrose to a concentration of 400 μg chlorophyll/ml. Thylakoids were phosphorylated by illumination (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) in the presence of 0.4 mM [τ-^{32}P]ATP (300000 cpm/nmol ATP). Alternatively, intact thylakoids were phosphorylated in the dark for 120 min by 1 mM NADPH and 10 μM ferredoxin. At different times of phosphorylation aliquots of thylakoids were removed and digitonin was added to a final concentration of 0.4%. The detergent solubilization was terminated after 2 min by a 10-fold dilution of the samples with incubation buffer at 0 °C. The homogenates were centrifuged at 40000 $\times g$ for 30 min. The supernatant, containing the stroma thylakoid fraction, was collected by centrifugation at 100000 $\times g$ for 60 min.

For heat treatment, thylakoids were suspended in 10 mM sodium phosphate buffer (pH 7.4), 5 mM NaCl, 5 mM $MgCl_2$, 100 mM sucrose and kept at different temperatures (40–50 °C) for 12 min. At different times during the heat-treatment samples of thylakoids were fragmented in a Yeda press at a nitrogen pressure of 17.5 MPa and separated into stroma lamellae and inside-out vesicles by differential centrifugation and phase partition as described in [11]. The isolation of inside-out vesicles was accomplished in a single partition step at 3 °C using a phase system composed of 5.6% dextran T-500, 5.6% poly(ethylene glycol) 3350, 10 mM sodium phosphate buffer (pH 7.4), 5 mM NaCl, 20 mM sucrose.

Quantification of the apopolypeptides of LHC II was done by two-dimensional gel electrophoresis (Fig. 1) as described in Ref. 19. For the first dimension chlorophyll-protein complexes were resolved by mild SDS-polyacrylamide gel electrophoresis [22]. For the second dimension the gree bands corresponding to LHC II (LHCP1 and LHCP3) were excised from the gel and reelectrophoresed separately under denaturing conditions in the buffer system of Laemmli [23]. The second-dimension gels were stained with Coomassie brilliant blue, destained and scanned using a laser densitometer. The relative amounts of the LHC II apopolypeptides were quantified from their peak areas.

In experiments using [τ-^{32}P]ATP, the second dimension gels were sliced, solubilized in H_2O_2/perchloric acid and counted for radioactivity in Aquasol.

Results

Phosphate incorporation of the 27 and 25 kDa polypeptides of LHC II

In a previous study we showed that upon illumination of spinach thylakoids for 10 min the 25 kDa polypeptide of LHC II has about three times as high specific phosphate incorporation as the 27 kDa polypeptide [19]. Here we show that also the phosphorylation kinetics of the two LHC II polypeptides are very different (Fig. 2). For the

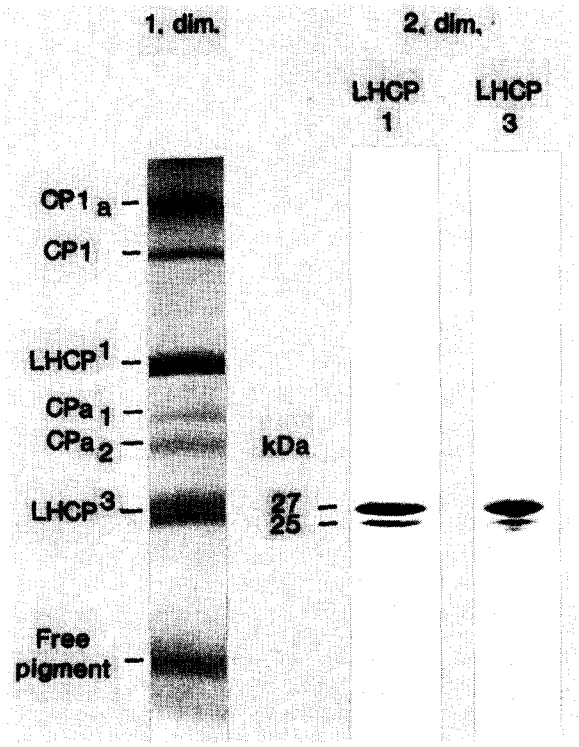


Fig. 1. Two-dimensional electrophores for resolution of LHC II apopolypeptides. In the first dimension chlorophyll-proteins were resolved mainly according to Ref. 22. For the second dimension the LHCP1 and LHCP3 band were excised and reelectrophoresed under denaturating conditions. The LHC II bands were identified by coelectrophoresis with the purified complex (not shown). The LHCP1 and LHCP3 polypeptides were quantified separately from gel scans.

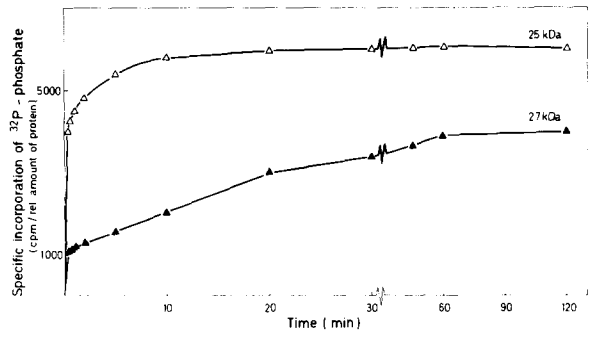
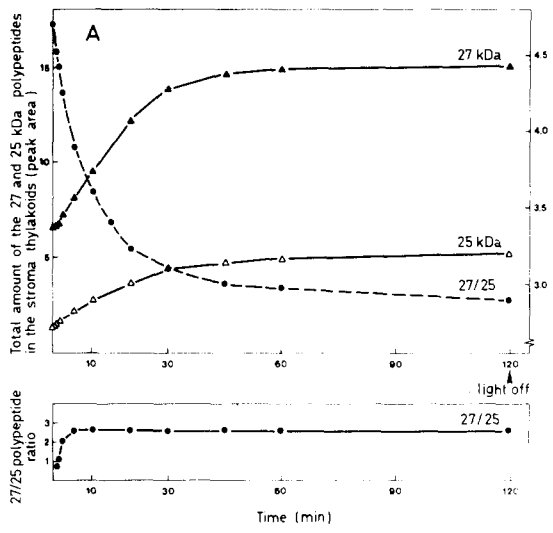


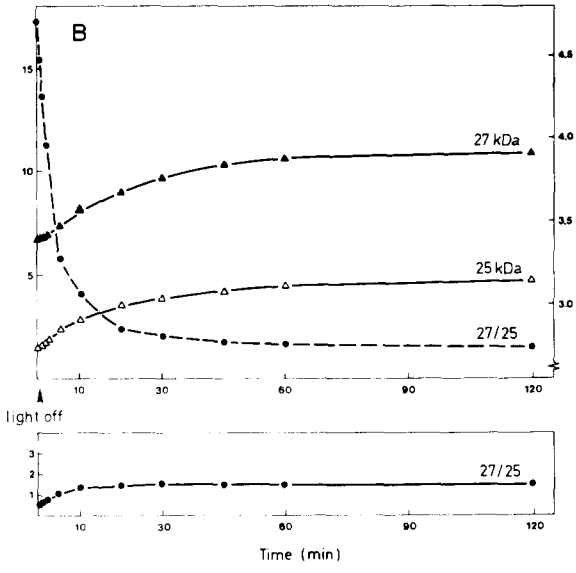
Fig. 2. Time-dependent specific incorporation of [^{32}P]phosphate into the 27 kDa (▲) and 25 kDa (△) polypeptides of LHC II in intact thylakoids during illumination for 120 min. The amounts of the 27 and 25 kDa polypeptides were quantified by two-dimensional gel electrohoresis. The second dimension gel was stained with Coomassie brilliant blue, destained and scanned. The relative amounts of the 27 and 25 kDa polypeptides were quantified from their peak areas. The total incorporation of [^{32}P]phosphate was analyzed after cutting out the individual apopolypeptide bands from the second-dimension gel. The specific phosphate incorporation (cpm/relative amount of protein) was determined after normalizing the total phosphate label to the corresponding amount of protein.

25 kDa polypeptide two-thirds of the maximal specific incorporation of [^{32}P]phosphate (cpm/relative amount of protein) is reached within 20 s of illumination and maximal phosphorylation requires only some 10 min. In the case of the 27 kDa polypeptide the rapid phosphorylation phase only brings about 25% of the maximal incorporation and full phosphorylation requires at least 60 min of illumination. The different phosphorylation kinetics of the two polypeptides can also be expressed in terms of the times for half maximal phosphorylation. Whereas the 27 kDa polypeptide has a $t_{1/2}$-value of 10 min, the 25 kDa polypeptide has reached the half maximal incorporation within a few seconds. It should also be noted that the maximal specific phosphorylation of the 27 kDa polypeptide never reaches the value for the 25 kDa polypeptide even after prolonged illumination.

Lateral migration of the 27 and 25 kDa polypeptides of LHC II following phosphorylation in the light

How does the different phosphorylation kinetics of the 27 and 25 kDa polypeptides relate to the lateral migration of LHC II from the appressed to the nonappressed thylakoid regions? The ratio between the 27 and 25 kDa polypeptides in the thylakoid membrane is 4.0 [19]. In isolated stroma thylakoids this ratio decreases upon phosphorylation from 4.2 to 3.2, demonstrating that the pool of phospho-LHC II exported to the nonappressed membranes upon phosphorylation is relatively enriched in the 25 kDa polypeptide compared to the bulk-LHC II. In this study we have further analysed the polypeptide composition of the migrating LHC II polypeptides. Fig. 3A (top) shows the time-course for the migration of the 27 and 25 kDa polypeptides into the nonappressed thylakoid regions during 120 min of illumination. At different times of illumination digitonin was added to an aliquot of the thylakoids, and subsequently stroma lamellae vesicles were isolated by





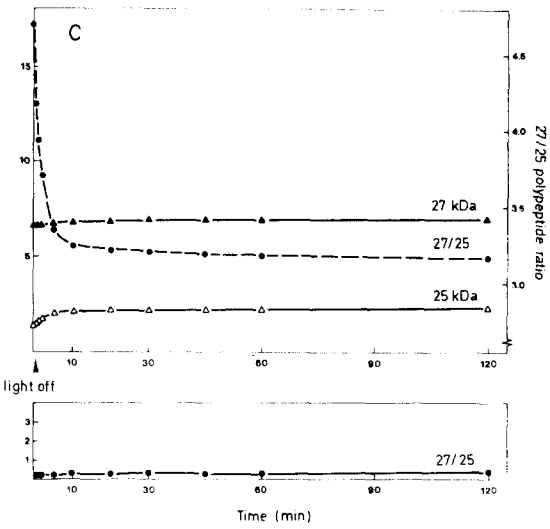


Fig. 3. Top: (A) Total amounts of the 27 kDa (▲) and 25 kDa (△) polypeptides of LHC II in the stroma thylakoids during illumination for 120 min in 5 mM $MgCl_2$. At different times of phosphorylation, intact thylakoids were fractionated by addition of digitonin and stroma lamellae vesicles were isolated by differential centrifugation. The amounts of the 27 and 25 kDa polypeptides were determined by their peak areas after two-dimensional gel electrophoresis. (B, C) Total amounts of the 27 kDa (▲) and 25 kDa (△) polypeptides of LHC II in the stroma thylakoids after illumination for only 20 s followed by 120 min of darkness in (b) 5 mM $MgCl_2$ or (c) 10 mM $MgCl_2$. At different times during the dark period stroma lamellae vesicles were isolated and the amounts of the 27 and 25 kDa polypeptides were determined as described above. In the figures (A–C) are also included the polypeptide ratios between the 27 and 25 kDa polypeptides in the stroma thylakoids at different times of incubation (- - - - - -). Arrows indicate when the light was switched off. Bottom: (A–C) Polypeptide ratios of the phospho-LHC II imported to the stroma thylakoids, calculated from the total amounts of the 27 and 25 kDa polypeptides in the stroma thylakoids at different times of incubation minus the amount of the polypeptides existing in the control stroma thylakoids (●).

differential centrifugation. The amount of total LHC II in the stroma thylakoids increases by 150% and there is a time-dependent increase of both polypeptides. However, the ratio between the 27 and 25 kDa polypeptides in the stroma thylakoids changes continuously, and after 120 min of illumination the ratio has dropped from 4.7 to 2.9. The 27/25 polypeptide ratio of the LHC II imported to the stroma thylakoids following phosphorylation was calculated to 2.5 (Fig. 3A, bottom) and with exception for the first minutes the ratio was constant throughout the illumination period. These data show that there is a time-dependent increase of the 25 kDa polypeptide relative to the 27 kDa polypeptide in the stroma thylakoids during phoshporylation and the low 27/25 polypeptide ratio of imported LHC II confirm our previous study that the migrating pool of LHC II is relatively enriched in the 25 kDa protein. However, when comparing Fig. 3A (top) with Fig. 2, the migration behaviours of the two polypeptides do not show a strict correlation with their phosphorylation kinetics. Whereas the $t_{1/2}$-values for the [^{32}P]phosphate incorporation of the 27 and

25 kDa polypeptides differ markedly, the $t_{1/2}$-values for the lateral migration of the two polypeptides in this experiment are about the same. If there were a correlation between the phoshporylation and migration behaviours, one should not expect a constant polypeptide ratio in the migrating pool of LHC II, but rather a slowly increasing 27/25 polypeptide ratio with longer times of illumination, since the 27 kDa polypeptide shows a slow phosphate incorporation.

To separate the phosphorylation and migration events of the LHC II and to maximize the difference in phosphorylation between the 27 and 25 kDa polypeptides, the illumination/subfractionation experiment was modified. The illumination period was reduced to only 20 s and then digitonin was added at various times during the following 120 min dark period. This very short period of illumination gives a predominate phosphorylation of the 25 kDa polypeptide even though the 27 kDa polypeptide also gets some minor incorporation (Fig. 2). No further [^{32}P]phosphate was incorporated in the proteins after the light was switched off. As seen in Fig. 3B the amount of 25 kDa polypeptides exported to stroma thylakoids is almost the same as in the previous experiment. In contrast, the relative increase of the 27 kDa polypeptide is only 60% compared to 130% at the longer time of illumination. The 27/25 polypeptide ratio of the stroma thylakoids drops to about 2.9 in 20 min to reach finally a value around 2.7. Thus, the 27/25 ratio of the imported phospho-LHC II is as low as 1.5 (Fig. 3B, bottom). Interestingly, the population of LHC II arriving to the stroma thylakoids during the first 2 min after the light was turned off has more 25 kDa polypeptides than 27 kDa polypeptides (a 27/25 polypeptide ratio of 0.6).

The experiments described above were made at 5 mM $MgCl_2$. In order to see if an increased screening of the negative phosphate ions (thought to drive the lateral migration of phospho-LHC II [24]) influenced the relative migration behaviour of the 27 and 25 kDa polypeptides, the experiment of Fig. 3B was repeated at 10 mM $MgCl_2$. At this relatively high concentration of divalent cations the increase of total LHC II in the stroma thylakoids was only 15% compared to 90% at 5 mM $MgCl_2$. As can be seen in fig. 3C (top), at 10 mM $MgCl_2$ there is very little lateral migration of the 27 kDa polypeptide into the stroma thylakoids. In contrast, there is a significant increase of the 25 kDa polypeptide. The ratio between the 27 and 25 kDa polypeptides in the migration pool of phospho-LHC II is as low as 0.3 (Fig. 3C, bottom), i.e., there is about three 25 kDa polypeptides for every 27 kDa polypeptide. The experiment was also tried at 15 mM $MgCl_2$ but no lateral migration of phospho-LHC II occurred at this higher level of Mg^{2+}.

It should be stressed that in none of the experiments described above we could detect any increase of Photosystem II core polypeptides or of the 29 kDa chlorophyll *a/b*-protein (CP29) in the stroma thylakoids. The CP29 does not even become phosphorylated (Larsson, U.K., unpublished results). This demonstrates the specificity of the lateral migration following phosphorylation and argue against that the observed changes in the content of the stroma lamellae vesicles could be explained simply by unstacking.

Lateral migration of the 27 and 25 kDa polypeptides of LHC II following phosphorylation in the dark by NADPH/ferredoxin

Thylakoid protein phosphorylation in the presence of NADPH and ferredoxin allows a higher incorporation of phosphate into LHC II compared to light-mediated phosphorylation. In our spinach thylakoids 120 min of illumination gives an average incorporation of 5 nmol ^{32}P/mg chlorophyll, whle NADPH/ferredoxin mediated phosphorylation gives 10 nmol ^{32}P/mg chlorophyll for the same period. The increased phosphate incorporation induced by NADPH/ferredoxin is reflected in a 220% increase of total LHC II in the stroma thylakoids which should be compared to a maximal increase of 150% after illumination. Fig. 4 (top) shows the migration behaviour of the 27 and 25 kDa polypeptides after phosphorylation in the presence of NADPH/ferredoxin during 120 min. For the first 30 min the migration of LHC II resembles very much that seen in the illumination experiment of Fig. 3A with an import into the stroma thylakoids of phospho-LHC II, possessing a 27/25 polypeptide ratio of 2.5 (Fig. 4, bottom). However, after 30 min of phosphorylation the 27/25 ratio of the

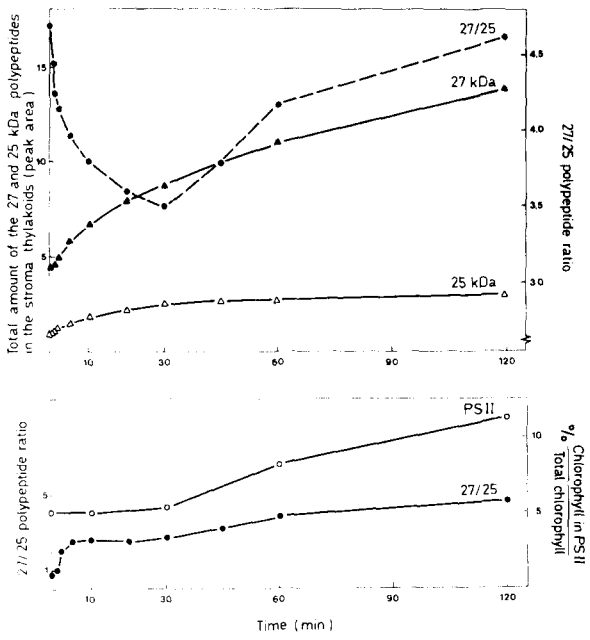


Fig. 4. Top: Total amounts of the 27 kDa (▲) and 25 kDa (△) polypeptides of LHC II and the 27/25 polypeptide ratios in the stroma thylakoids at different times of phosphorylation in the dark. Intact thylakoids were phosphorylated for 120 min by 1 mM NADPH and 10 μM ferredoxin in 5 mM $MgCl_2$. At different times of phosphorylation the thylakoids were fractionated by addition of digitonin and the stroma lamellae fractions were isolated. The amounts of the two apopolypeptides of LHC II were quantified from their peak areas after two-dimensional gel electrophoresis. Bottom: The calculated 27/25 polypeptide ratios of LHC II (●) and the relative amounts of Photosystem II (○) imported to the stroma thylakoids. Quantification of Photosystem II was done by analyzing the relative proportion (%) of the chlorophyll-protein cmplex of Photosystem II (CPa) resolved by mild SDS-polyacrylamide gel electrophoresis [22].

LHC II imported to the stroma thylakoids starts to increase and finally reaches a value around 4.6. Looking at the specific phospho-LHC II arriving to the stroma thylakoids after 30 min of phosphorylation, the 27/25 polypeptide ratio is as high as 10. Strikingly, concomitantly with the arrival of phospho-LHC II with such a high 27/25 ratio there is an increase in the amount of the Photosystem II core in the stroma thylakoids (Fig. 4, bottom). Thus, after 120 min of phosphorylation the amount of the chlorophyll *a*-protein complex of Photosystem II in the stroma thylakoids, resolved as the CPa bands on an SDS-polyacrylamide gel electrophoresis [22], increased from 5% to 11%. These observations suggest that under conditions of high-phosphate incorporation also the Photosystem II core complex, which contains some subunits able to undergo phosphorylation [25], can leave the appressed thylakoid regions in favour of the stroma thylakoids. The coordinated migration behaviour of an LHC II pool rich in the 27 kDa polypeptide and the Photosystem II core strongly suggests that the pool of LHC II that is closely associated with the Photosystem II core contains predominantly the 27 kDa protein.

Lateral migration of the 27 and 25 kDa polypeptides of LHC II at elevated temperatures

In addition to phosphorylation elevated temperatures can induce a detachment of LHC II from the Photosystem II core as revealed from freeze-fracture [12] and subfractionation [11] studies. After such a temperature-induced dissociation Photosystem II and a closely associated pool of LHC II migrate to the stroma thylakoids, while a pool of free LHC II remains in appressed thylakoid regions. How do these subpopulations of LHC II relate to those seen after phosphorylation particularly with respect to their relative contents of the 27 and 25 kDa polypeptides? To this end we have analyzed the amount of the two polypeptides in appressed and nonappressed thylakoid membranes after isolation of inside-out and stroma lamellae vesicles from heat-treated and control thylakoids. Fig. 5 shows the 27/25 polypeptide ratios of LHC II in inside-out vesicles isolated after different times of incubation of thylakoids at 40, 45 and 50 °C. At all three temperatures there is a time-dependent decrease in the 27/25 ratio of the LHC II in the appressed thylakoids. The decrease is most pronounced at 50 °C. Thus, the 27/25 polypeptide ratio of the free LHC II remaining in the appressed thylakoids is about 2.4–2.6, a value close to that observed for the pool of LHC II migrating to the stroma thylakoids under normal phosphorylation conditions (Fig. 3A). The ratio between the 27 and 25 kDa polypeptides in the Photosystem II-associated pool of LHC II migrating to the stroma thylakoids has a value around 4.5–5 (Fig. 5). This ratio is also obtained for the small pool of LHC II residing in the control stroma thylakoids (Fig. 3). We therefore conclude that the two subpopulations of LHC

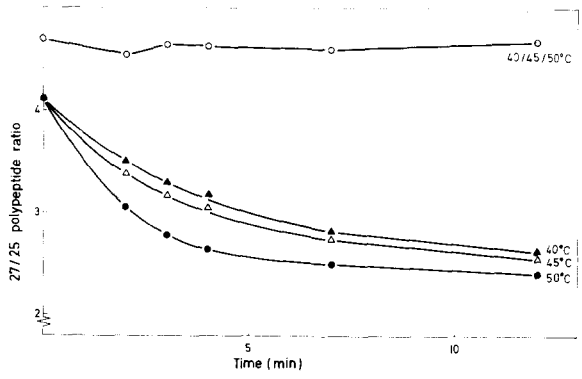


Fig. 5. Time-course of the ratio between the 27 and 25 kDa polypeptides of LHC II in inside-out vesicles isolated from thylakoids incubated at 40 °C (▲), 45 °C (△) and 50 °C (●). Included are also the 27/25 polypeptide ratios of isolated stroma thylakoids at all three temperatures (○). At different times of incubation the thylakoids were fractionated in a Yeda press and inside-out and stroma lamellae vesicles were collected by differential centrifugation and phase partition. The relative amounts of the two apopolypeptides were quantified from their peak areas after two-dimensional gel electrophoresis.

II that dissociate or remain connected to the Photosystem II core after heat treatment or phosphorylation are identical.

It should be noted that neither the phosphorylation nor the heat treatents themselves altered the proportion between the two polypeptides in unfractionated thlakoids. Thus, the changes seen in the 27/25 polypeptide ratio do not reflect changes in the overall content of the two polypeptides but are rather a consequence of lateral reorganizations in the antenna system of Photosystem II.

Discussion

Our analyses of the polypeptide composition of LHC II in subfractions isolated from phosphorylated or heated thylakoids provide evidence for the existence of two subpopulations of LHC II. One population is more closely bound to the Photosystem II core and contains a high proportion of the 27 kDa polypeptide. The other population, containing a relatively high proportion of the 25 kDa polypeptide, is more peripherally bound to the core and is able to detach from photosystem II reversibly due to phosphorylation or to increased temperatures. The pool of LHC II that migrates to the stroma thylakoids following normal phosphorylation or that remains in the appressed thylakoids as free LHC II at high temperatures belongs to this peripheral subpopulation of LHC II. Lateral migration of the inner or tightly bound LHC II occurs only under conditions when there is a lateral migration of the Photosystem II core, i.e., at heavy phosphate incorporation (Fig. 4) or at elevated temperatures (Fig. 5). Our identification and characterization of two different subpopulations of LHC II probably relate to the bound and mobile pools of LHC II postulated from ultrastructural studies on phosphorylated thylakoids [7].

The detailed polypeptide composition and organization of the two LHC II subpopulations is not quite clear. Studies on the heat-induced lateral rearrangement of the antenna system of Photosystem II suggest that the two LHC II pools are about equally abundant in the thylakoid membranes [11]. We propose that the inner LHC II contains mainly or maybe even only the 27 kDa polypeptide, while the peripheral pool contains both the 27 and 25 kDa polypeptides in a proportion of 2 : 1. This would give a 27/25 polypeptide ratio of approx. 4 for the bulk-LHC II in unfractionated spinach thylakoids. Analyses of the ratio between the two polypeptides in the stroma thylakoids after heat treatment (Fig. 5) show that the LHC II population that is moving together with the Photosystem II core at elevated temperatures has the same relative amount of the 27 kDa polypeptides as the small pool of LHC II present in control stroma thylakoids. This suggests that Photosystem II of the stroma thylakoids, likely to be Photosystem II_β [26,27] contains mainly the 27 kDa polypeptide in its LHC II antenna. Some 25 kDa polypeptides are seen both in control stroma thylakoids as well as in LHC II migrating together with the Photosystem II core. At present we cannot judge whether these 25 kDa polypeptides are physically connected to the Photosystem II-bound pool of LHC II or are due to the presence of free peripheral LHC II units.

The peripheral pool of LHC II may have some heterogeneous properties. Although this population normally was found to have a 27/25 polypeptide ratio around 2, we could under conditions of low phosphate incorporation observe the migra-

tion of a very small LHC II pool with a ratio around 0.5. Since LHC II subunits are believed to be arranged in trimers [28], the peripheral pool could be composed of two types of heterotrimer, one with two 25 kDa polypeptides for every 27 kDa polypeptide and the reversed proportion in the other type of trimer. Alternatively, the obtained ratios could be explained by mixtures of 27 and 25 kDa polypeptide homotrimers. At present we cannot judge from our two-dimensional gel electrophoresis whether the 27 kDa polypeptides of the peripheral and inner LHC II pools are identical. Moreover, we cannot exclude the possibility of further polypeptide heterogeneity of LHC II which then would complicate the arrangement discussed above.

What is the mechanism behind the migration behaviour of the Photosystem II core and the different LHC II pools under the various phosphorylation conditions or at the elevated temperatures? As shown by Barber [24], the lateral distribution of protein complexes along the stacked thylakoid membrane is controlled by a balance between their surface charges and the ionic conditions. In addition, Allen and Holmes [29] have emphasized the importance of mutual electrostatic repulsion parallel to the plane of the membrane between phosphoproteins. Applied to the migration behaviour of the LHC II apopolypeptides the latter model predicts that lateral migration of the 25 kDa phosphopolypeptide may require also phosphorylation of the 27 kDa polypeptide. This would be consistent with our observation that although the 25 kDa polypeptide is very rapidly phosphorylated compared to the 27 kDa polypeptide (Fig. 2), the migration kinetics is much slower than expected (Fig. 3A) and its migration seems to be dependent on the slow phosphorylation of the 27 kDa polypeptide.

Fig. 6 gives a model for the heterogenous and dynamic properties of the antenna of Photosystem II. The interaction between the peripheral and inner subpopulations of LHC II is suggested to be comparatively weak. As a result of mutual electrostatic repulsion between neighbouring phosphorylated polypeptides this binding will easily break and the two subpopulations will dissociate. At elevated temperatures there is no need for introduction of negatively charged phosphate groups to

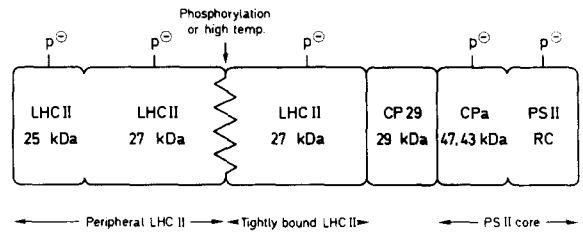


Fig. 6. Model of the heterogeneous and dynamic organization of the light-harvesting antenna of Photosystem II. The comparatively weak interaction between the peripheral and tightly bound (inner) subpopulations of LHC II will break as a result of repulsive charges appearing upon phosphorylation or elevated temperatures and the two LHC II pools will dissociate (RC = reaction centre of Photosystem II).

achieve this dissociation. Instead the repulsive forces contributed by the charged amino acids themselves become dominating when the Van der Waals attraction is weakened with the increased temperatures. Why is there no dissociation of the tightly bound pool of LHC II from the Photosystem II core although both entities become phosphorylated? We suggest that this is due to the presence of a non-phosphorylated protein located between the core and the LHC II, thereby acting as a 'charge spacer'. An obvious candidate for such a protein would be the 29 kDa chlorophyll *a/b*-protein (CP 29). This protein has been suggested to be close to the Photosystem II core [30], does not become phosphorylated (Larsson, U.K., unpublished observation) and stays with the core both under conditions of phosphorylation [31] and elevated temperatures [11]. Thus, CP 29 would not contribute to any repulsive charges enabling the tightly bound subpopulation of LHC II to be released from the Photosystem II core despite both being phosphorylated.

Our experiment also demonstrates that the balance between protein surface charges and cation level is crucial for the phosphorylation-induced lateral migration. Under high Mg^{2+} concentration the total amount of phospho-LHC II exported to the stroma thylakoids is low and this migrating pool of LHC II contains a very high abundance of the rapidly phosphorylated 25 kDa subunit (Fig. 3C). Moreover, at very high phosphate incorporation at 5 mM Mg^{2+} even the phosphorylated Photosystem II core with its tightly associated

LHC II antenna is able to migrate (Fig. 4). This has been shown to occur also under normal phosphorylation conditions and lowered concentration of Mg^{2+} [32]. However, the events taken place at high phosphorylation and/or low Mg^{2+} concentrations can, at least in part, be explained by unstacking rather than lateral migration of phosphoproteins.

Our structural results are in agreement with recent fluorescent data suggesting that changes in the absorption cross-section of the photosystems correlates with the phosphorylation of a 'mobile' LHC II pool enriched in the low-molecular-weight polypeptide [20]. Further phosphorylation was found to induce 'spill-over' between the photosystems.

At present we do not have any explanation for the mechanism behind the migration behaviours of Photosystem II and LHC II at elevated temperatures which is completely reverse to the situation at phosphorylation.

The present work provides support for a structural and functional heterogeneity in addition to the previously known heterogeneity of LHC II at the gene and protein levels [13–15]. It will be a task for future research to investigate the control mechanisms for the synthesis of the different LHC II polypeptides and to what extent this is related to the multigene family coding for LHC II. Interestingly, our recent results suggest that the peripheral pool of LHC II is responsible for the long-term variation in the antenna size of Photosystem II in response to different light conditions [33].

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