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Fragmentation and separation of the thylakoid membrane Effect of light-induced protein phosphorylation on domain composition

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Abstract

The thylakoid membrane consists of three main compartments, the stroma lamellae, the central appressed region of grana and the peripheral grana margins. These three domains can be separated from each other by fragmentation and aqueous two-phase partitioning. Upon phosphorylation the light-harvesting chlorophyll *a*/*b* binding complex II (LHC II) dissociates away from photosystem II in grana and migrates into the stroma lamellae. Dynamic processes, such as lateral migration of LHC II, can be examined by analysing corresponding fragments from phosphorylated and non-phosphorylated thylakoids. Our results show that we can detect not only the well studied lateral migration of phosphorylated LHC II from grana to stroma lamellae, but also migration within the grana disc, from the core of grana to the margins of grana. Furthermore, we show that the grana margin vesicles and the stroma lamellae vesicles are heterogeneous populations that can be fractionated further by counter-current distribution.

1. Introduction

The photosynthetic membrane in higher-plant chloroplasts is thought to be a single-membrane continuum, organized into grana stacks and intergranal connections of stroma-exposed membranes, the so called stroma lamellae. The membrane consists of several domains that have probably evolved in order to carry out specialized functions. The main function of the thylakoid membrane is to capture light quanta and to convert the light energy into chemical energy. The light energy is used to drive a series of redox reactions whereby water is oxidized to protons and oxygen while ferrodoxin is reduced concomitantly with the synthesis of ATP.

Protein complexes involved in the light reactions of photosynthesis, such as α and β forms of photosystems I and II, are segregated laterally in the membrane continuum. The thylakoid membrane can be fragmented by mechanical means such as press treatments or sonication. The fragments so obtained are in the form of membrane vesicles which can be separated by a combination of centrifugation and aqueous two-phase partitioning. Thus, sonication of thylakoid membranes followed by centrifugation and partitioning by aqueous two-phase systems gives rise to many membrane fragments of differing properties. Based on earlier work [1–4] we have shown that the thylakoid membrane consists of

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at least three domains, the stroma lamellae, the central appressed region of grana and the peripheral grana margins. Fragments derived from domains of the thylakoid membrane can yield valuable information for a better understanding of the light reaction of photosynthesis.

Proteins are known to migrate between domains within the thylakoid membrane due to changes in environmental conditions such as light intensity. Regulation of the light-harvesting antenna of photosystem (PS) II by phosphorylation of the light-harvesting chlorophyll *a*/*b* binding complex (LHC II) causes detachment of LHC II from PS II. Phosphorylated LHC II is then believed to migrate away from PS II into PS I-enriched domains [5–7] but whether or not the redirected excitation energy can be trapped by PS I has not been unequivocally established. Migration of phosphorylated LHC II from grana into PS I-rich domains has been explained by different mechanisms such as protein–protein electrostatic forces [7,8] or by thermal diffusion once LHC II is detached from PS II [9].

In this study we use a rapid procedure to fractionate the thylakoid membrane into vesicle populations deriving from grana and stroma lamellae. By fractionating the grana-derived vesicles further by sonication and partitioning by means of an aqueous two-phase system we obtain vesicles representing grana core and grana margins. By comparing these vesicle populations from phosphorylated and control thylakoids we are able to investigate lateral migration of proteins within the grana stacks, that is from the core of granum to the margins of granum.

2. Materials and methods

2.1. Chemicals

Dextran T500 was obtained from Pharmacia (Uppsala, Sweden). Poly(ethylene glycol) (PEG) 4000 (Carbovax PEG 3350) was supplied by Union Carbide (New York, NY, USA). [γ-^{32}P]ATP was prepared as described [10].

2.2. Chloroplast isolation

Spinach chloroplasts were isolated from dark adapted leaves, osmotically broken and washed as described in ref. 2 except the final wash which was carried out in medium comprising 10 m*M* sodium phosphate buffer (pH 7.4)/5 m*M* NaCl/10 m*M* $MgCl_2$/5 m*M* NaF/100 m*M* sucrose. The thylakoids were resuspended in the same medium to give a concentration of about 1 mg chlorophyll/ml prior to illumination.

2.3. Light-induced protein phosphorylation

Phosphorylation took place in the above mentioned medium by illuminating thylakoids in the presence of 0.1 m*M* ATP for 15 min at 18°C under continuous stirring. Incubation took place under red light (630–680 nm, 200 μE m^{-2} s^{-1}). Control thylakoids were illuminated in absence of ATP. When samples for phosphorimaging were prepared phosphorylation was performed as described above except that 50 μCi/ml [γ-^{32}P] ATP was also added. After the illumination, samples were washed twice in the illumination medium and resuspended in the same medium to a chlorophyll concentration of about 4 mg/ml.

2.4. Fragmentation of the thylakoid membrane

Thylakoid suspension (2 g) was added to 9.66 g of a polymer mixture to give 5.8% (w/w) Dextran T500/5.8% (w/w) PEG 4000/10 m*M* sodium phosphate buffer (pH 7.4)/5 m*M* NaF/2 m*M* $MgCl_2$/20 m*M* sucrose. After 45 min incubation on ice the thylakoids were sonicated in a Vibra-cell ultrasonic processor, Model VC 500 (Sonics and Materials, Danbury, CT, USA) equipped with a 1/2-in. (1 in. = 2.54 cm) horn. Thylakoids were sonicated six times for 30 s with resting intervals of 1 min in a cylindrical aluminium tube immersed in ice. The ultrasonic exposure had an intensity output setting of 7, with 20% duty pulses. After sonication 6.43 g lower phase and 5 g top phase (composition of lower and top phase as described below) were

added to the sonicate. This then constituted the "sample system".

2.5. Separation of membrane fragments by phase partitioning

Vesicles derived from grana and stroma lamellae were separated by a batch procedure in three steps [2] or in a quantitative way by counter-current distribution at 4°C. In both procedures the composition of the phase system used for the separation of grana vesicles from the stroma lamellae vesicles, and the grana core vesicles from the grana margin vesicles was always 5.8% (w/w) Dextran T500/5.8% PEG 4000/10 m*M* sodium phosphate buffer (pH 7.4)/20 m*M* sucrose/10 m*M* NaF. For counter-current distribution an apparatus where the phase separation is speeded up by centrifugation was used [11]. The apparatus contains 60 cavities, cavities 0–2 were loaded with 1.8 ml "sample system" and cavities 3–59 by 1.8 ml phase system (0.9 ml top phase and 0.9 ml lower phase). One operation cycle comprised 30 s mixing followed by 90 s centrifugation and one transfer. Normally a total of 55 cycles were carried out. When phosphorylated and control vesicles were compared the respective samples were loaded in three cavities on opposite halves of the plate and only 25 operation cycles carried out.

Preparation of grana core and grana margins vesicles was carried out by sonicating the grana vesicles further. The lower phase from the batch procedure containing the grana derived vesicles was sonicated sixteen times for 30 s, under the same conditions and using the same settings as described above for the sonication of whole thylakoids, then fractionated by counter-current distribution.

Further fractionation of the grana margin vesicles and the stroma lamellae vesicles was carried out without sonication in phase systems of higher polymer concentrations. The phase systems contained 6.0% (w/w) of both polymers (Dextran T500 and PEG 4000) for fractionation of the grana margin vesicles but 6.2% (w/w) of both polymers (Dextran T500 and PEG 4000) for separation of the stroma lamellae-derived vesicles, otherwise the phase systems had the same composition as the phase system previously described.

After fractionation the vesicles were released from the polymers by two-fold dilution in a medium comprised of 100 m*M* sucrose/10 m*M* sodium phosphate buffer/10 m*M* NaF and absorbance at 680 and 650 nm recorded for each cavity. Fractions were then pooled in order to collect peaks of interest and pelleted by centrifugation at 100 000 *g* for 90 min. Pellets were then resuspended in the dilution medium and dimethyl sulphoxide added to yield 5% (w/v) when vesicles were to be stored in liquid nitrogen.

2.6. Analysis

Measurements of chlorophyll concentration and the determination of chlorophyll *a*/*b* ratio of the various samples was carried out according to Arnon [12].

Absorbance difference measurements for the quantification of P_{700} (reaction centre of PS I) were performed according to ref. 13 using a DW-2 Aminco spectrophotometer operating in a split-beam mode. The concentration of P_{700} was determined from the amplitude of the light–dark absorbance change at 700 nm, using an extinction coefficient of 64 mM^{-1} cm^{-1}.

Kinetic measurements of P_{700} photooxidation were carried out according to ref. 14, and the potassium cyanide treatment of isolated membrane vesicles according to ref. 15 using 150 m*M* KCN for 2 h. A DW-2 Aminco spectrophotometer, working in dual-wavelength mode with 700 nm as measuring wavelength and 730 nm as reference, was used for the kinetic measurements. The optical path-length of the cuvette was 10 mm. The actinic beam was transmitted by a 380–600-nm broad band filter, a 566.9-nm interference filter HBW 80, and attenuated by a neutral filter to give an uniform light between 500 and 600 nm and an intensity of 25 μE m^{-2} s^{-1}. Nicolet NIC Model 527 was used for signal averaging.

Cytochrome *f* content was determined immu-

nologically by rocket immunoelectrophoresis [16] at pH 8.6. The concentration of cytochrome *f* standard (Sigma) was determined spectroscopically.

Sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis was performed according to the method of Laemmli [17], using a 12–22.5% polyacrylamide gradient slab gel containing 4 *M* urea.

Measurements of ^{32}P-labeled polypeptides in SDS gels were performed by phosphorimaging with a Fuji BAS 2000 Bio-Imaging Analyzer.

3. Results

The schematic model of the thylakoid membrane shown in Fig. 1a represents a structure thought to be a single-membrane continuum with different domains. Fig. 1b illustrates the proposed domain organization within the grana stacks with PS IIα in the central core of the granum and PS Iα at the margins of granum. It is assumed that upon light-induced phosphorylation, LCH II is detached from PS IIα and migrates into PS I-rich domains.

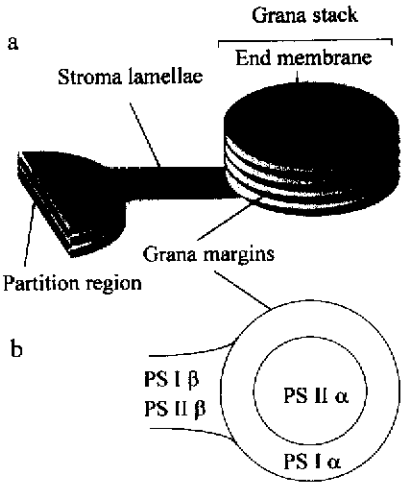

Fig. 1. Model for organization of the thylakoid membrane. (a) Different domains of the membrane. (b) Proposed domain organization within the grana stack with PS IIα in the core of granum and PS Iα in the margins of the granum. PS Iβ and PS IIβ with their smaller antennae are located in stroma lamellae.

3.1. Fractionation strategy

Fig. 2 illustrates the fractionation strategy. Thylakoids are first fragmented by sonication and separated into grana (α) and stroma lamellae (β) vesicles (Fig. 2a). By further sonication of the grana vesicles their margins can be peeled off resulting in two, occasionally three, well separated peaks in the counter-current distribution diagram (Fig. 2b). The peak labeled α3 represents the margin fraction while the vesicles

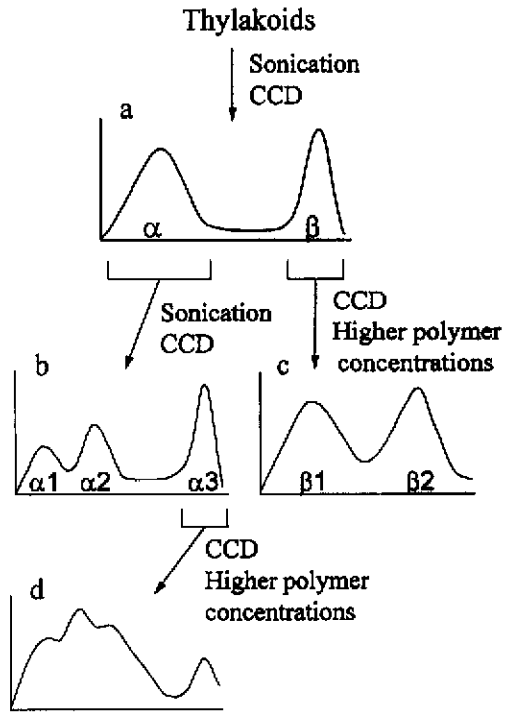

Fig. 2. Fractionation strategy demonstrating: (a) Separation of grana (α) and stroma lamellae vesicles (β) by counter-current distribution (CCD). (b) Sub-fractionation of the grana vesicles, margins of grana labeled α3 and the core of grana, fractions labeled α1 and α2. (c) Separation of stroma lamellae vesicles and (d) separation of grana margin vesicles in phase systems of higher polymer concentrations. Ordinates represent absorbance at 680 nm and abscissas fractions 1–60 from the counter-current distribution plate.

of the $\alpha 1$ and $\alpha 2$ peaks originate from the central core of the grana [3,18]. The margin vesicle population ($\alpha 3$) can be further separated without additional sonication in a phase system of higher polymer concentration (Fig. 2d) giving rise to three or four peaks. Likewise the stroma lamella vesicles (b) can be separated into two sub-populations $\beta 1$ and $\beta 2$ (Fig. 2c).

3.2. Fractionation of thylakoids

Counter-current distribution of sonicated thylakoids is shown in Fig. 3a. Individual cavities were pooled to obtain nine fractions for further analysis (see bar at the top of Fig. 3a). Chlorophyll a/b ratios, P_{700} content and polypeptide pattern (not shown) confirm that stroma lamellae vesicles are in the peak to the right. The grana peak is quite heterogeneous, chlorophyll a/b ratios range from 2.2 to 2.7 (see bar at the top of Fig. 3a).

Fig. 3b is a plot of P_{700} *vs.* cytochrome f from the pooled fractions. The P_{700} content varies from 0.9 to 4.2 (mmol/mol chlorophyll) while the content of cytochrome f varies from 2.8 to 3.5 (mmol/mol chlorophyll). The non-linear relationship amongst these data indicate the existence of at least three different pools of cytochrome f as demonstrated in ref. 19.

The value for the kinetic constant for P_{700} photooxidation and thereby also the antenna size of PS I in the different fractions is decreasing with higher chlorophyll a/b ratio (Fig. 3c). It shows that the PS I of the two peaks differ in antenna size. A 20 to 30% larger antenna size is found in the population originating from grana as compared to the population originating from the stroma lamellae. Others [20] have reported an even larger antenna size difference (40%) between these PS I populations of the grana and stroma lamellae vesicles.

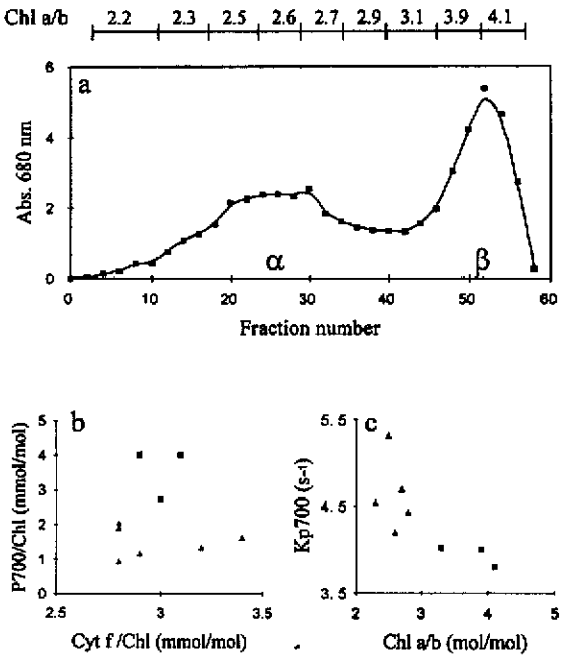

Fig. 3. Heterogeneity within the grana and stroma lamellae populations. (a) Counter-current distribution of sonicated thylakoids. For each of the nine pooled fractions (see bar at the top) the content of cytochrome (Cyt) f and P_{700} was determinated as also the kinetics of P_{700} photooxidation. Chlorophyll (Chl) a/b ratios for nine pooled fractions shown at the top. (b) P_{700}/Chl as a function of Cyt f/Chl for the pooled fractions. (c) The rate constant, KP_{700}, for photooxidation of P_{700} as a function of chlorophyll a/b ratio for the pooled fractions. Triangles in b and c represent fractions from the grana peak (α) and squares stand for fractions from the stroma peak (β) in Fig. 3a.

3.3. Subfractionation of grana lamellae

When the grana vesicles (α) are collected, sonicated and then fractionated by counter-current distribution a diagram as seen in Fig. 4a is obtained; $\alpha 1$ and $\alpha 2$ represent the grana core with low chlorophyll a/b ratio (2.2–2.3) and large PS II antennae size; $\alpha 3$ represents the grana margins with higher chlorophyll a/b ratio (2.6), and more PS I as compared to the core fractions. Further fractionation of the grana margin fraction ($\alpha 3$) in a phase system of higher polymer concentration containing 6.0% (w/w) of the polymers Dextran T500 and PEG 4000 gives rise to three or four new peaks (Fig. 4b). These populations derived from the grana margin region have slightly different chlorophyll and polypeptide compositions (see bar at the top of Fig. 4b for the chlorophyll a/b ratios).

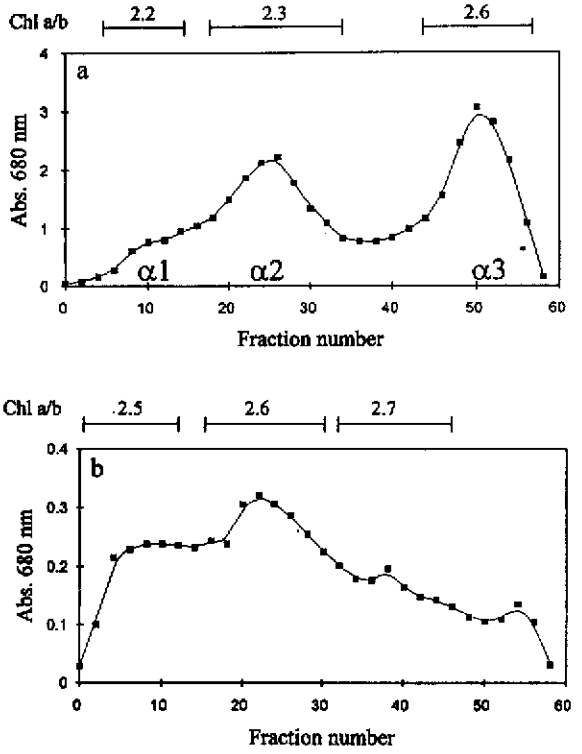

Fig. 4. (a) Counter-current distribution of sonicated grana (α) vesicles. The peak to the right (α3) consists of grana margin vesicles and the peaks to the left consist of grana core vesicles (α1 and α2). (b) Counter-current distribution of grana margin vesicles (α3) in a phase system of higher polymer concentrations.

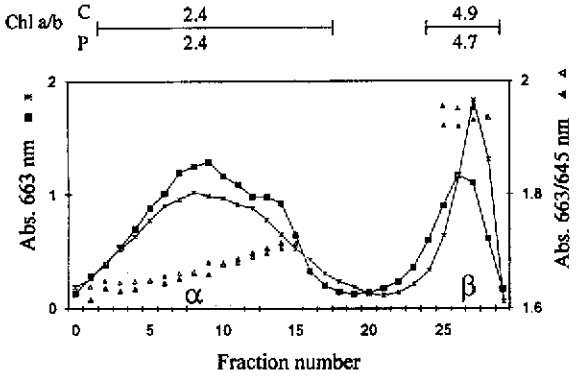

Fig. 5. Counter-current distribution of phosphorylated and non-phosphorylated sonicated thylakoids. Absorption at 663 nm for (■) non-phosphorylated thylakoids and (*) phosphorylated thylakoids. Absorption ratio 663/645 nm for (▲) non-phosphorylated thylakoids and (△) phosphorylated thylakoids.

3.4. Fractionation of phosphorylated thylakoids

In the counter-current distribution diagram for sonicated phosphorylated thylakoids (Fig. 5) more material is obtained on a chlorophyll basis in the stroma lamellae fraction (β) compared to the non-phosphorylated stroma fraction. Furthermore, the chlorophyll *a*/*b* ratio of the phosphorylated stroma lamellae fraction is lower. In contrast, the control (non-phosphorylated) and the phosphorylated grana fractions have very similar chlorophyll *a*/*b* ratios. A closer examination of all the tubes under the α peak (grana) shows, however, a significant variation in the chlorophyll *a*/*b* ratio as revealed by the ratio between the light absorption at 663 and 645 nm.

The lower chlorophyll *a*/*b* ratio for the phosphorylated stroma lamellae fraction can be explained by lateral movements of phosphorylated LHC II, with low chlorophyll *a*/*b* ratio (about 1.0), from the grana lamellae to stroma lamellae or by partial destacking of grana stacks resulting in the formation of more non-appressed membrane vesicles during sonication. Concomitant increase in the chlorophyll *a*/*b* ratio for the grana fractions is not seen which can be explained by the fact that the chlorophyll *a*/*b* ratio of LHC II (about 1.0) is closer to that of the grana fraction (2.4) compared to the chlorophyll *a*/*b* ratio for the stroma lamellae fraction (4.9) and also that the loss of membrane material from the α peak is relatively less than the concomitant gain in the β peak (Fig. 5). The loss of phosphorylated LHC II does therefore not contribute significantly to an increase in the chlorophyll *a*/*b* ratio of the phosphorylated grana fraction.

The α peaks of both the phosphorylated and non-phosphorylated samples are heterogeneous with respect to the chlorophyll *a*/*b* ratios (ranging from 2.2 to 2.7 for different pooled fractions as seen in Fig. 3a.) This heterogeneity might be explained by assuming that vesicles containing relatively more grana margins are present in the right-hand part of the α peak whereas vesicles in the left-hand part consist of vesicles more rich in the partition region from which most of the margins have been peeled off by sonication.

The two plots 663/645 nm for control and phosphorylated thylakoids cross at fraction 8 under the α peak in Fig. 5. In fractions 1–7 the phosphorylated fractions have a higher chlorophyll a/b ratio than their controls while the opposite holds for fractions 9–14. This can be explained by lateral migrations of phosphorylated LHC II from the centre into the margins of grana due to its phosphorylation (see Discussion below). The experiment of Fig. 5 has been repeated two times and the 663/645 plots cross under the α peak in both cases. It should be stressed that a counter-current distribution with 25 transfers (Fig. 5) involves 25 separate partitions and therefore errors of individual partitions are cancelled to a great extent. The difference between the two 663/645 plots of Fig. 5 is therefore significant even though it is small.

When the stroma lamellae vesicles from phosphorylated and control thylakoids are fractionated by counter-current distribution in a phase system of higher polymer concentration, without further sonication, two peaks are obtained (Fig. 6). For the control β vesicles the peak to the left has higher chlorophyll a/b ratio than the peak to the right in the counter-current distribution diagram. In the case of phosphorylated vesicles the peaks have similar chlorophyll a/b ratio but more material is found in the peak to the right as compared to control β vesicles. Both peaks from the phosphorylated membrane have lower chlorophyll a/b ratios than their corresponding control fractions. This shows that more LHC II is found in both stroma lamellae populations $\beta 1$ and $\beta 2$ after phosphorylation.

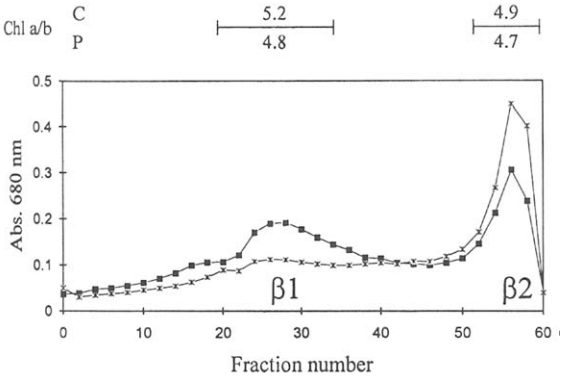

Fig. 6. Counter-current distribution of stroma lamellae (β) vesicles. (■) = Non-phosphorylated (β) vesicles; (*) = phosphorylated (β) vesicles.

3.5. Imaging and analysis of phosphoprotein heterogeneity

The phosphorimage (Fig. 7) shows that ^{32}P-labeled PS II polypeptides [CP 43, D1,D2 and 9 kDa (*i.e.* M_r 9000)] are predominantly found in the grana derived fractions, grana core and grana margins. Two additional polypeptides of apparent molecular mass 62 000 and 64 000 are found most labeled in the grana fractions, where slightly more labeling is found in the grana margin fraction compared to the grana core fraction. These polypeptides may represent the M_r 64 000 protein kinase associated with the cytochrome b/f complex [21,22] which is in agreement with previous results where the M_r 64 000 protein kinase is found to be enriched in the grana margins [21–23]. ^{32}P-Labeled LHC II polypeptides are found rather evenly distributed throughout the membrane.

Analyses of ^{32}P-labeled PS II polypeptides and LHC II polypeptides quantified from SDS gels (Fig. 7) are shown in Fig. 8 a, c and d. In Fig. 8a photo-stimulated luminescence (PSL) for CP 43

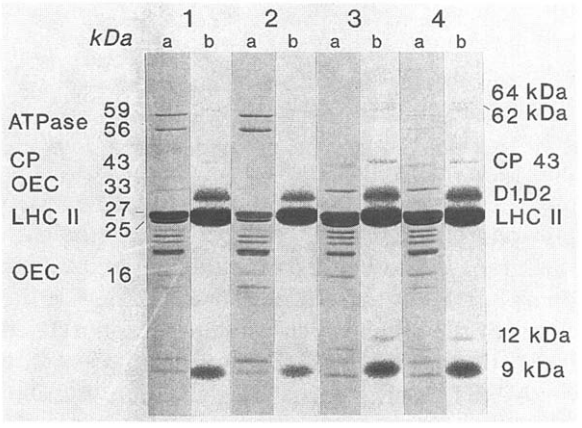

Fig. 7. SDS–polyacrylamide gel electrophoresis analysis (lanes a) and subsequent phosphorimages (lanes b) of fragments from phosphorylated thylakoid membrane: 1 = thylakoids, 2 = stroma lamellae, 3 = grana core and 4 = grana margins. The same amount of chlorophyll (17 mg) was loaded in each lane. kDa = kilodalton.

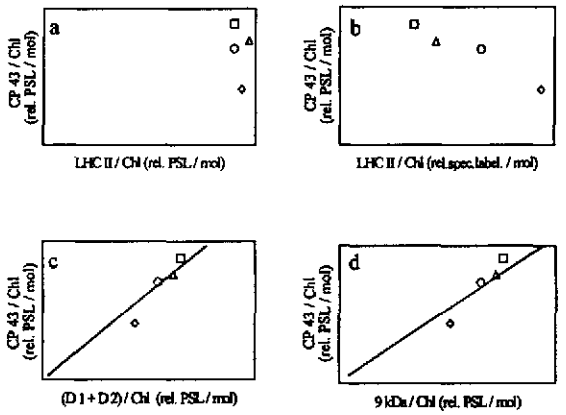

Fig. 8. Relation between ^{32}P-labeled thylakoid proteins for the different fractions estimated from SDS gels (Fig. 7). □ = Grana core; △ = grana margins; ○ = thylakoids; ◇ = stroma lamellae. Photostimulated luminescence (PSL) obtained for CP 43 as a function of: (a) PSL obtained from LHC II polypeptides, (b) specific labeling of LHC II polypeptides, (c) PSL obtained from D1 and D2 polypeptides and (d) PSL obtained from the M_r 9000 (9 kDa) phosphoprotein of PS II.

is plotted against PSL obtained for LHC II which gives a straight vertical line showing that almost the same amount of ^{32}P-labeled LHC II is found in all the fractions. In Fig. 8b PSL for CP 43 is plotted against specific labeling of LHC II (the relative amount of LHC II was estimated from the peak area after scanning of the Coomassie brilliant blue-stained gels). The plot shows linear relationship with a negative slope, *i.e.* the specific labeling of LHC II decreases with increased labeling of CP 43. This means that phosphorylated light-harvesting complexes II migrate from grana to stroma lamellae whereas phosphorylated CP 43 polypeptides remain in the grana. Another explanation might be that phosphorylation causes partial destacking at the edges of the grana which contain phosphorylated LHC II and PS Iα and that these areas are removed from the grana by sonication and end up in the stroma lamellae fraction. Fig. 8c and d show that there is a linear stochiometric relationship between the labeled PS II polypeptides CP 43, D1 + D2 and M_r 9000 phosphoprotein in the different membrane domains. This shows that under our conditions no detectable movement of any one individual PS II polypeptide away from the PS II complex into other domains is observed.

4. Discussion

The thylakoid membrane consists of three main compartments, the stroma lamellae, the central appressed region of grana and the peripheral grana margins. These three domains can be separated from each other by fragmentation and aqueous two-phase partitioning [24]. It has been shown that those photosystem II units with large antennae, PS IIα, are localized in the grana partitions while those PS II centres with smaller antennae, PS IIβ, are localized in the stroma lamellae and grana margins [3]. The PS I units with large antennae, PS Iα, are localized in the grana margins while PS Iβ having smaller antennae are localized in the stroma lamellae [2,3]. The cytochrome *b/f* complex is found to be uniformly distributed within the thylakoid membrane but in varying concentrations, highest in the grana partitions and lowest in the grana margins [19]. The ratio between PS IIα and cytochrome *b/f* in the partition region is about 2 to 1 and these two complexes may form a super complex in the membrane [25]. The ATP synthase is found only in the unappressed regions, *i.e.* in the stroma lamellae and the grana margins.

This work shows that these three main domains of the thylakoid membrane consist of subdomains. The reason for this conclusion is that upon further fragmentation and separation, or by only further separation by counter-current distribution in a new phase system, the vesicle populations are found to be heterogeneous, giving rise to distinct peaks in the counter-current distribution diagram. For example, the margin vesicle fraction designated α3 in Fig. 4a is separated into three, probably four different sub-populations having different chlorophyll *a/b* ratios, and the stroma lamellae fraction (β in Figs. 2a or 3a) splits up into two separate peaks (Fig. 6) also having different chlorophyll *a/b* ratios.

It has been shown that the grana vesicles (α) are inside-out while the stroma lamellae vesicles (β) are right side out [26]. It is probably this

difference in sideness which contributes to the efficient separation of these two types of vesicles. The grana margin vesicles ($\alpha 3$), which are peeled off from grana core by sonication, have a right side out conformation [3] and are separated efficiently from the remaining inside-out vesicles originating from the centre of the grana. The mechanism behind the separation of the different stroma lamellae vesicles (Fig. 6) or margin vesicles (Fig. 4b) which are all right side out is however not known to us. It may reflect the number of ATP synthase or LHC II complexes, exposed on the surface of the vesicles.

Light-induced protein phosphorylation results in a significant effect on the counter-current distribution patterns. The peak representing grana vesicles (α peak in Fig. 5) becomes smaller and the peak representing the stroma lamellae vesicles (β peak in Fig. 5) becomes larger than the control peak. This may be due to partial unstacking of the grana stacks whereby some of the grana margins, which are enriched in PS Iα, (*i.e.* more LHC II is bound to PS I in grana margins compared to PS I in stroma lamella) are peeled off and turned right-side out and collected in the β peak. Alternatively some LHC II may have migrated from the grana to the stroma lamellae as has been suggested to occur upon phosphorylation [5–7]. Both explanations are supported by the lower chlorophyll *a*/*b* ratio of the β peak after phosphorylation.

Of particular interest is the shift in the chlorophyll *a*/*b* ratio in the various fractions under the α peak of Fig. 5 (fractions 1–15). This shows that in fractions 1–8 the chlorophyll *a*/*b* ratio goes up upon phosphorylation while the opposite change occurs in fractions 9–14. It is reasonable to assume that the fractions 9–14, because of their higher chlorophyll *a*/*b* ratios, contain vesicles relatively rich in grana margins. Margin vesicles have a chlorophyll *a*/*b* ratio of 2.6–3 compared to a ratio of 2.0–2.2 for the vesicles originating from the partion region [3]. Hence the reason for the decrease in the chlorophyll *a*/*b* ratio of fractions 9–14 and corresponding increase for fractions 1–8 upon phosphorylation may be due to migration of LHC II, with its low chlorophyll *a*/*b* ratio, from the central core of the grana disc to the peripheral margins. If this interpretation is correct then this is the first time a migration of LHC II from the centre of the grana to the margins has been demonstrated. Since it has been suggested [1,20] that the linear electron transport occurs in the grana, between PS IIα and PS Iα, it is reasonable to suggest that a migration of LHC II within the grana is important for the regulation of non-cyclic electron transport.

Also in the case of stroma lamellae the phosphorylation has a significant effect on the counter-current distribution pattern (Fig. 6). Here the relative size of the two peaks change after phosphorylation. The largest shift in the chlorophyll *a*/*b* ratio occurs in the peak to the left of Fig. 6. This might mean that the left peak represents vesicles which originate from a special domain of the stroma lamellae which receives selectively more LHC II from the grana.

Taken together our results indicate that there is, after phosphorylation of the thylakoid membrane proteins, a movement of LHC II from the centre of the grana into both the margins of grana and into the stroma lamellae. This may be relevant for the regulation of the photosynthetic electron transport, both non-cyclic in the grana and cyclic in the stroma lamellae.

This work demonstrates the usefulness of fragmentation in combination with aqueous two-phase partitioning for analysis of both the domain organization of the thylakoid membrane and dynamic processes within this membrane. The same approach described here should, with slight modifications, also be applicable to other biological membranes. [27]

5. Acknowledgements

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6. References

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