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Low temperature effects on thylakoid protein phosphorylation and membrane dynamics

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The effective antenna size of Photosystem II is regulated by a kinase mediated phosphorylation of the main light-harvesting chlorophyll *a/b* protein (LHCII). This regulatory mechanism, which involves lateral migration of the phospho-protein along the thylakoid membrane, is thought to be essential for short term acclimation of the photosynthetic light harvesting. In this study we have analyzed how LHCII phosphorylation and the subsequent changes in the organization of the thylakoid membrane are influenced by low temperatures. It was shown that the kinase activity, measured as degree of LHCII phosphorylation, is operational at 0°C although partially inhibited. By subfractionation of thylakoid membranes phosphorylated at 0°C it was shown that virtually no phospho-LHCII migrates to the stroma thylakoids at this low temperature, in contrast to the case at normal temperatures. When such thylakoids, with phospho-LHCII retained in the appressed grana regions, were gradually subjected to increasing temperatures followed by subfractionation, it was shown that rapid lateral migration of phospho-LHCII was induced in a quite narrow temperature range of 10–12°C. At 5°C the migration of phospho LHCII would require hours for completion while at 20°C all phospho-LHCII had arrived in the stroma thylakoids within 5 min. From a functional point of view, our results reveal that at temperatures when the migration of phospho-LHCII from the grana region is prevented, there is no reduction in the effective antenna size of Photosystem II. This shows that protein phosphorylation in itself is not sufficient to create a functional disconnection between Photosystem II and LHCII but that the subsequent lateral diffusion of phospho-LHCII in the thylakoid membrane is required. The significance of the results in connection with increased photoinhibition during combined light and cold stress is discussed. Apart from these physiological implications, the present combination of protein phosphorylation and thylakoid subfractionation offers a novel way to study lateral diffusion of a single protein in an undisturbed biomembrane.

Introduction

Plants have to acclimate to a variety of different environmental conditions for optimal function and survival. Many of these acclimation mechanisms are manifested at the molecular level involving transcriptional, translational and posttranslational regulations [1,2]. Two major stress factors to plants are high light condi-

Abbreviations: LHCII, light-harvesting chlorophyll *a/b*-protein complex of Photosystem II; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol.

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tions low temperatures which are particularly harmful if they are combined [3,4]. Often such a combined light and cold stress leads to severe photoinhibition due to impairement of Photosystem II.

A molecular mechanism that is regarded as essential for the short term regulation of the light-harvesting of the photosynthetic apparatus in algae and higher plants is protein phosphorylation [5–8]. A membrane bound kinase [7,9] phosphorylates the main light-harvesting chlorophyll *a/b*-protein complex of Photosystem II (LHC II) in addition to several subunits of the Photosystem II core [7]. The kinase activity is controlled by the redox state of electron transport components such as plastoquinone [5,10] and the cytochrome *b/f* complex [11–14] which function between the two photosystems. As a result of the protein phosphorylation one subpopulation of LHC II dissociates from Photosystem II and migrates along the membrane away from the appressed thylakoids into the Photosystem I rich non-appressed thylakoid regions [6,8,15–17]. This rearrangement in the organization of the light-harvesting antenna, which requires a fluid membrane bilayer, has been suggested to be important for the balancing of excitation energy between the two photosystems [5–7] as well as protecting against photodamage of Photosystem II [8,18].

In order to understand the molecular basis for light-cold stress on photosynthesis we have made an in vitro study on the effect of low temperature on protein phosphorylation and the accompanying lateral rearrangements in the thylakoid membrane. The results show that the protein kinase is functional at low temperatures but that the rapid lateral migration of phospho-LHCII in the thylakoid membrane is largely prevented below 10°C, and that there is no decrease in the antenna size of Photosystem II. The results are discussed in biochemical terms with respect to thylakoid membrane fluidity and lateral diffusion of integral membrane proteins and in physiological terms in relation to combined light and cold stress of plants.

Materials and Methods

Spinach was grown hydroponically in nutrient solution [19] at 25°C and 475 μmol photons m^{-2} s^{-1}. Thylakoids were isolated according to Ref. 20 and finally suspended in 50 mM Tricine-KOH (pH 7.6), 20 mM NaCl, 5 mM $MgCl_2$ and 100 mM sorbitol (incubation buffer). For protein phosphorylation, the thylakoids were suspended in the incubation buffer at a chlorophyll concentration of 0.4 mg/ml and kept in darkness at room temperature for 10 min before being brought to the desired temperature. 10 mM NaF and 0.4 mM ATP containing [γ-^{32}P]ATP (0.035 mCi/mg chlorophyll) were added to the suspension which was then illuminated (500 μmol photons m^{-2} s^{-1}) for 10 min. Alternatively, protein phosphorylation was performed with the same additions in the dark for 5 min in the presence of 1 mM NADPH and 10 μM ferredoxin. The reaction was stopped either by addition of Laemmli solubilization buffer or digitonin (see below), depending on the nature of the experiment.

Scheme I.

Subfractionation at various temperatures of thylakoid membranes phosphorylated at 0°C.

1. Protein phosphorylation of intact thylakoids at 0 C in the presence of [γ-^{32}P] ATP.
2. Incubation in the dark at different temperatures (0°C–20°C).
3. Withdrawal of samples for digitonin based subfractionation at specified timepoints.
4. Isolation of stroma (or grana) membranes.
5. Analysis of phospho-LHCII content by SDS-PAGE and autoradiography or scintillation counting.

Grana and stroma thylakoid membranes were prepared by digitonin based subfractionation of stacked thylakoids essentially according to Ref. 21. One volume of 0.8% digitonin was added to the thylakoid suspension (0.4 mg chlorophyll/ml) giving a final concentration of 0.4% digitonin. The solubilization at 20°C was stopped after 2.5 min by adding ten volumes of ice-cold incubation buffer. The grana membranes were isolated by centrifugation at $10000 \times g$ for 5 min. The resulting supernatant was spun at $40000 \times g$ for 30 min and the stroma thylakoids contained in the supernatant were finally collected by centrifugation at $100000 \times g$ for 60 min.

In order to follow any changes in the lateral location of LHCII subsequent to protein phosphorylation, as a function of temperature and time, the following 'kinetic' subfractionation experiment was designed (Scheme I). After the thylakoids had been subjected to protein phosphorylation at 0°C the samples were divided into different lots. Each of these were transferred to a defined temperature in the 0°C–20°C range. At specific time-points at each temperature, samples were withdrawn and subfractionated into grana and stroma thylakoids by the digitonin method.

SDS-PAGE was carried out according to [22] using a 12 to 22.5% polyacrylamide gradient in the separation gel. Prior to electrophoresis the samples were suspended in the Laemmli solubilization buffer [22] and incubated at 70°C for 5 min. The gels were autoradiographed and the films subsequently scanned by laserdensitometry for quantification.

Photosystem II electron transport was measured at 3°C in a Hansatech oxygraph using light passed through a 650 nm interference filter. The light intensity in the range of 14 to 2680 μmol photons m^{-2} s^{-1} was modulated by neutral density filters. Phenyl-*p*-benzoquinone (0.5 mM) was used as an artificial electron acceptor.

The assay medium consisted of 25 mM Hepes-NaOH (pH 7.5), 10 mM NaCl, 2.5 mM $MgCl_2$ and 100 mM sucrose. Thylakoids were added to a final concentration of 20 μg chlorophyll/ml. Chlorophyll concentration was determined in 80% acetone according to Arnon [23].

For electron microscopy, thylakoids were quickly spun down, resuspended in 50 mM sodium phosphate (pH 7.3) and fixed in 2.5% glutaraldehyde. The samples were postfixed in 1% osmium tetroxide in phosphate buffer for 2 h at room temperature and subsequently washed, dehydrated in an acetone series and embedded in Spurr's resin. Ultrathin sections were cut on a Sorvall Ultra Microtome MT 5000. The sections were stained with uranyl acetate and lead citrate and observed using a Zeiss EM 10 electron microscope.

Results

The effect of low temperatures on protein phosphorylation and the subsequent organizational and functional changes in the photosynthetic apparatus was investigated in vitro using isolated spinach thylakoids.

Initially, the temperature dependence of the kinase mediated phosphorylation of LHCII was investigated. Fig. 1 depicts an experiment where the degree of phosphorylation at different temperatures was determined. It shows that whether light or NADPH/ferredoxin was used to activate the kinase, the level of LHCII phosphorylated was almost as high at 0°C as it was at 20°C. This indicates that the kinase is not particularly sensitive to low temperatures. It should be pointed out, however, that the results were variable and in some thylakoid preparations the kinase activity at 0°C was only 25% of that at 20°C. The reason for this variation is not yet clear, but the experiments show that phosphorylation of LHCII indeed takes place at temperatures as low as 0°C.

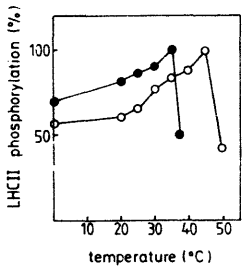

Fig. 1. Temperature dependence of LHCII phosphorylation in isolated thylakoids. ●, 5 min light mediated protein phosphorylation; ○, phosphorylation 5 min in the dark mediated by NADPH and ferredoxin. Total [^{32}P] phosphate incorporation was determined by scintillation counting of bands excised from SDS-PAGE. 100% refers to maximum labelling. (20000 cpm and 30000 cpm, respectively.)

TABLE I

[^{32}P]Phosphate incorporation in LHCII in thylakoids and thylakoid subfractions isolated after protein phosphorylation in intact thylakoids at 0°C or 22°C

The specific [^{32}P]phosphate incorporation was calculated by normalizing the total phosphate label to the corresponding amount of protein quantified from the peak areas of the Coomassie-stained SDS-polyacrylamide gels.

Fraction	Amount of label in LHCII temperature	
	0°C	22°C
Intact thylakoids	5300	5565
Grana thylakoids	5150	5500
Stroma exposed thylakoids	285	4730

If the temperature was raised above 20°C, a relatively sharp increase in the degree of phosphorylation was observed, particularly when NADPH/ferredoxin was used as reducing agent (Fig. 1). This increase in protein phosphorylation was followed by a sudden drop at 35°C, in the case of the light-induced reaction, or at approx. 45°C in the case of the NADPH/ferredoxin induced phosphorylation. In the case of the light induced phosphorylation, the curve probably primarily reflects the effect of temperature on the photosynthetic electron transport and its ability to reduce the plastoquinone pool. Hence, the temperature dependence observed with NADPH/ferredoxin most likely represents a more direct measure of the heat stability of the kinase activity.

Although lowered temperatures in most cases did not severely affect the protein kinase activity per se, they may drastically influence the subsequent lateral migration of phospho-LHCII [8], considering its dependence on membrane fluidity. In order to investigate this, thylakoid membranes phosphorylated at different temperatures, were subfractionated into grana and stroma thylakoids, using digitonin incubation and differential centrifugation. The subfractionation method was devised so that intermediate organizational changes of the phosphorylated thylakoid membranes at defined temperatures and time intervals could be trapped and detected (Scheme I). In Table I it can be seen that when thylakoids were phosphorylated at 0°C, radiolabel was almost exclusively found in the grana membranes. In contrast, when the same experiment was performed at room temperature, grana and stroma lamellae had the same relative degree of LHCII phosphorylation. These observations strongly indicate that the kinase is situated only in the appressed regions of the stacked thylakoid membrane and that phospho-LHCII cannot migrate to the non-appressed regions at 0°C. This view is further strengthened by measurements showing that stroma lamellae vesicles isolated from thylakoids phosphorylated at 0°C have the same

chlorophyll a/b ratio (6.6–6.8) and relative LHCII content (11%) as those isolated from unphosphorylated control thylakoids.

This subfraction analysis suggests that phosphorylated LHCII is trapped in the appressed regions of grana at low temperatures. In order to see if this leads to any rearrangements in the grana structure, i.e., destacking, control thylakoids and thylakoids phosphorylated at 0°C and subsequently kept at 0°C or transferred to 20°C, respectively, were analyzed by electron microscopy (Fig. 2). The ultrastructural appearance of the three samples was very much the same. When the number and the sizes of grana stacks were calculated from several representative micrographs, no differences could be found between non-phosphorylated thylakoids and thylakoids phosphorylated and kept at 0°C or 20°C. This suggests that no destacking occurs in response to thylakoid protein phosphorylation at 0°C.

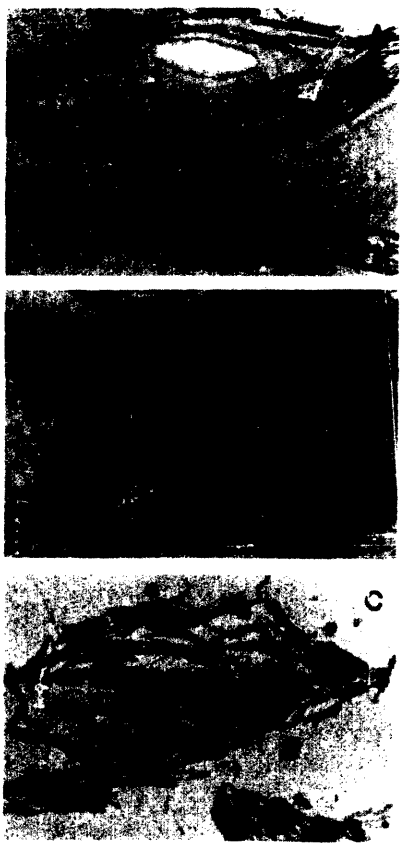

Fig. 2. Electron micrographs of (A) unphosphorylated control thylakoids; and (B) and (C) thylakoids phosphorylated at 0°C and subsequently kept at 0°C (B) or at 20°C. Bar represents 1 μm.

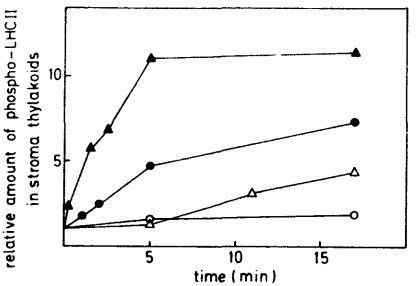

Fig. 3. Time dependence of the appearance of phospho-LHCII in isolated stroma thylakoids at different temperatures. After protein phosphorylation at 0°C, thylakoids were incubated for the indicated times in the dark at 0°C (○); 5°C, (△); 12.5°C (●); 20°C (▲) before isolation of stroma thylakoids.

To test if the phosphorylated LHCII molecules, trapped in the grana appressions, would start to migrate once the temperature was raised, thylakoids were phosphorylated at 0°C and thereafter incubated at different increasing temperatures for various times prior to fractionation (Scheme I). As illustrated in Fig. 3, the rate of appearance of phospho-LHCII in the stroma vesicles is strongly dependent upon temperature. At 20°C the first LHCII molecules appeared very rapidly in the stroma membranes, and after 5 min the total 'migrating population' of phospho-LHCII had already arrived (Fig. 3). In contrast, at 5°C the LHCII migration within 5 min was very limited and it would have taken more than 1 h for all mobile phospho-LHCII to arrive in the stroma membranes. At 0°C virtually no migration of phospho-LHCII was seen (Fig. 3). When initial rates of migration, are plotted against temperature (Fig. 4), a strong biphasic nature of the temperature dependence of the diffusion is observed. A marked increase in the diffusion rate is observed at temperatures above 10°C, indicating a 'critical migration temperature' around this temperature.

It has previously been shown in a large number of studies that phosphorylation of LHCII at room temperature results in a decrease in the size of the Photosystem II antenna [5,6,24–26]. Considering the results presented above, phosphorylation at low temperatures provided a means to test if the LHCII phosphorylation as such is responsible for this decrease or if the subsequent lateral movement is also required. In order to experimentally address this problem light saturation curves of Photosystem II dependent oxygen evolution

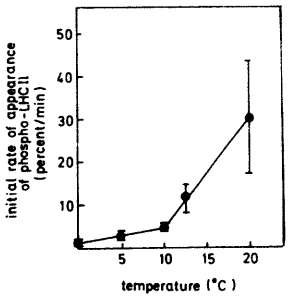

Fig. 4. Initial migration rates of phospho-LHCII as a function of temperature. Initial rates were calculated from several experiments equivalent to those in Fig. 3. Each point represents the mean of two to four experiments. The error bars show the standard deviation.

were determined for thylakoids phosphorylated at 0°C and subsequently kept at 0°C or transferred to 20°C for 20 min. The temperature in the oxygraph was kept at 3°C, o avoid lateral migrations during the assay, and hence relatively low rates of O_2 evolution were obtained. The data for electron transport rates were transferred into an Eadie-Hofstee plot (Fig. 5) in which the slope is equal to K_m, the light intensity required for half-maximal activity of oxygen evolution [26]. This value was taken as an estimate of the effective antenna size. From the data presented in Fig. 5 it can be

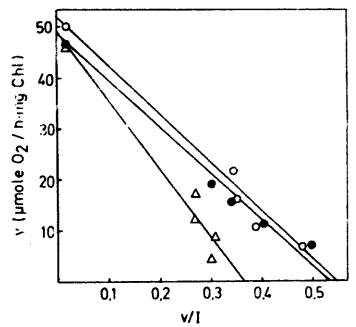

Fig. 5. Eadie-Hofstee plot of the light saturation curve of Photosystem II-dependent electron transport in phosphorylated thylakoids subjected to different temperatures. Thylakoids were phosphorylated in the light at 0°C and subsequently incubated in the dark for 20 min at 0°C (○) or 20°C (△). ●, control, unphosphorylated thylakoids kept in the dark at 0°C. v equals the O_2 evolution rate at different light intensities (I). (I) was varied between 14 and 2680 µmol photons m^{-2} s^{-1}.

calculated that when thylakoids were phosphorylated at 0°C and thereafter incubated at 20°C, the half saturation value was increased from 94 µmol photons m^{-2} s^{-1} to 130 µmol photons m^{-2} s^{-1}. This illustrates a reduction of the Photosystem II antenna of approx. 30% which is normally seen following LHCII phosphorylation at room temperature [26]. In contrast, the thylakoid sample phosphorylated at 0°C and kept at this low temperature showed the same K_m value (91 µmol photons m^{-2} s^{-1}) as in the unphosphorylated control thylakoids (Fig. 5), demonstrating that the effective antenna size of Photosystem II did not change. It can therefore be concluded that phosphorylation as such is not sufficient to create a functional disconnection between Photosystem II and LHC II but that removal of phospho-LHCII through lateral diffusion from the appressed into the stroma exposed thyladkoid region is also required.

Discussion

The phosphorylation of LHCII is considered to be essential for regulation and protection of the photosynthetic apparatus against imbalances in the light supply. The mechanism for this molecular acclimation is quite complicated, involving a redox mediated activation of kinase activity, protein–protein dissociation and lateral diffusion of phospho-proteins within the membrane lipid bilayer. In this study we have analyzed how low temperatures influence the phosphorylation of LHCII and the subsequent rearrangements in the organization of the thylakoid membrane. In part, the study has a physiological dimension since combined cold and light stress is particularly damaging to the photosynthetic apparatus [3,4]. Moreover, the study is of significance for understanding the temperature dependence of lateral migration of a single protein component within the thylakoid membrane.

LHCII polypeptides show a substantial phosphorylation close to freezing temperatures indicating that both the activation of the kinase and the enzymatic activity itself can tolerate low temperatures. In certain experiments we could detect as much as 80% of LHCII phosphorylation compared to the normal room temperature experiments.

Even though the kinase activity is operational at low temperatures, LHCII phosphorylation does not induce the organizational and functional alterations in the Photosystem II antenna (Figs. 3–5). It can therefore be concluded that it is not the protein phosphorylation itself that regulates the antenna size of photosystem II but that there is an absolute requirement for lateral migration of phospho-LHCII. This observation, in an undisturbed thylakoid membrane system, corroborates a study of Haworth [27] who incorporated cholesterol hemisuccinate into the thylakoid membrane, which did

not affect the kinase activity but which prevented the state I–state II transitions.

What significance does the lack of phosphorylation induced reduction in the Photosystem II antenna at low temperatures have on our understanding of the mechanism behind the increased light induced impairment of photosynthetic effiency in the cold [3,4]? In a recent in vitro study [28] we showed that the Photosystem II electron transport in itself is not more susceptible to photoinhibition at low temperatures. It was therefore argued that the synergistic stimulation of photoinhibition in vivo by light and low temperatures is not due to effects on the electron transport but rather due to reduced efficiency of protection and/or repair mechanisms. The inability of phospho-LHCII to functionally disconnect from Photosystem II below 10°C due to the restricted lateral mobility as shown in the present study gives strong support to the argument that reduced protection is a significant factor for combined light and cold stress. Indirectly, our study also gives support for diminished repair at low temperatures since restoration of photoinhibited Photosystem II requires lateral migration of damaged centres [29,30] and newly synthesized D1-protein from between the two thylakoids [31]. Interestingly, plants can be acclimated to low temperatures and be more resistant to light stress and photoinhibition [32]. A molecular understanding of this acclimation and its relation to thylakoid membrane fluidity and lateral migration of proteins is therefore an essential task for future experimentation. Preliminary studies in our laboratory indicate that the 'critical migration temperature' of phospho-LHCII is lower in spinach acclimated to cold temperatures.

Lateral migration and randomization of all membrane proteins take place during the reversible thylakoid destacking that occurs under low salt conditions [6,33]. It is well known from freeze-fracture analyses [33] and fluorescence measurements [34] that the lateral migration of membrane complexes in the salt-induced destacking/restacking process is highly temperature dependent.

Our present subfractionation procedure allows a detailed analysis of the lateral mobility of one single protein unit, the outer pool of LHCII, induced for migration in the thylakoid membrane by a physiologically regulated posttranslational phosphorylation. At low temperatures the migration is very slow, requiring hours for completion. However, there is a quite distinct increase in the lateral mobility of phospho-LHCII at temperatures around 10°C. Above this 'critical migration temperature' the extent and rate of LHCII migration into the stroma thylakoids rapidly reaches that seen at room temperature conditions. The reason for this biphasic nature of the mobility of LHCII is not obvious. The lipid bilayer of plant thylakoid membranes which is predominantly composed of galactolipids (MGDG and DGDG) with an unusually high degree of unsaturation [35], does not undergo any obvious gross phase changes over the temperature range of −20°C to 55°C [36].

Thus, the quite sharp increase in mobility of phospho-LHCII above 10°C can not be attributed only to properties of membrane lipids such as fatty acid unsaturation. This notion is experimentally supported by a spin label EPR study on cyanobacteria grown at different temperatures showing that changes in fatty acid composition does not influence the thylakoid viscosity [37]. However, Barber and co-workers have shown that plants grown at low temperatures possesses a more fluid thylakoid membrane than plants grown at higher temperatures [36]. Recent studies using spin label electron spin resonance spectroscopy emphasizes the role of lipid–protein interactions affecting the fluidity at different temperatures of the membrane [38]. Whether the distinct increase in lateral mobility of phospho-LHCII at 10–12°C is due to such a change in the interaction between lipids and proteins remains to be established.

Integral membrane proteins usually have a diffusion coefficient in the range of 10^{-9}–10^{-12} cm^2 s^{-1} with most proteins gathered in the upper part of this range. [39]. However, the estimation of such a diffusion coefficient is not easy and often involves specific modification of a protein using a chemical or immunological label followed by spectroscopically based analyses. Such approaches have their limitations in that they may induce pertubation of the protein or membrane structure. In that respect our present study offers a unique opportunity since it is based on lateral mobility in a undisturbed membrane where the migration has been induced by a physiological addition of a phosphate group at the exposed N-terminus of LHCII. Based upon the rate of appearance of LHCII in stroma thylakoids at 20°C (Fig. 3), Einstein's equation [40] can be used to calculate an approximative diffusion coefficient of 10^{-12} cm^2 s^{-1} for the phosphorylated protein, assuming that the radius of a granum is 300 nm. However, a strict analysis of the diffusion of phospho-LHCII would require a Monte-Carlo simulation as performed for other situations of lateral migration in the thylakoid membrane [41,42].

It is generally assumed that the migration of phospho-LHCII occurs from any part of the appressed thylakoid regions. However recent analyses based upon immuno-gold electronmicroscopy using an antibody against the LHCII-kinase suggest that the enzyme has a somewhat non-uniform distribution within the appressed regions with an enrichement in the outer portions close to the margins [43]. Thus migration of phospho-LHCII may mainly occur from areas close to the margins into the non-appressed regions. Such a situation would be consistent with the observation that

the LHCII antenna of Photosystem II is largest in the midportion of a granum and decreases towards the periphery [44].

The present subfractionation procedure should be useful for investigating the rate and temperature dependence of other protein diffusion processes occurring in the thylakoid membrane, such as remigration of dephosphorylated LHCII into the grana regions and movement of newly synthesized Photosystem II proteins from non-appressed to appressed membrane regions.

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References

1 Kuhlemeier, C., Green, P.J. and Chua, N.-H. (1987) Annu. Rev. Plant Physiol. 38, 221–257.
2 Anderson, J.M. (1986) Annu. Rev. Plant Physiol. 37, 93–136.
3 Öquist, G., Greer, D.H. and Ögren, E. (1987) in Topics in Photosynthesis (Barber, J., ed.), Vol. 9, pp. 67–87, Elsevier, Amsterdam.
4 Baker, N.R., Long, S.P. and Ort, D.R. (1988) in Plants and Temperature (Long, S.P. and Woodward, F.I., eds.), pp. 347–375, The Company of Biologists Ltd., Cambridge.
5 Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Nature, 291, 25–29.
6 Barber, J. (1982) Annu. Rev. Plant Physiol. 33, 261–295.
7 Bennett, J. (1983) Biochem. J. 212, 1–13.
8 Anderson, J.M. and Andersson, B. (1988) Trends Biochem. Sci. 13, 351–355.
9 Coughlan, S.J. and Hind, G. (1986) J. Biol. Chem. 261, 11378–11385.
10 Horton, P., Allen, J.F., Black, M.T. and Bennett, J. (1981) FEBS Lett. 125, 193–196.
11 Gal, A., Shahak, Y., Schuster, G. and Ohad, I. (1987) FEBS Lett. 221, 205–210.
12 Bennett, J., Shaw, E.K. and Michel, H.P. (1988) Eur. J. Biochem. 171, 95–100.
13 Wollmann, F.-A. and Lemaire, C. (1988) Biochim. Biophys. Acta 933, 85–94.
14 Coughlan, S.J. (1988) Biochim. Biophys. Acta 933, 413–422.
15 Andersson, B., Åkerlund, H.-E., Jergil, B. and Larsson, C. (1982) FEBS Lett. 149, 181–185.
16 Kyle, D.J., Staehelin, L.A. and Arntzen, C.J. (1983) Arch. Biochem. Biophys. 222, 527–541.
17 Larsson, U.K., Sundby, C. and Andersson, B. (1987) Biochim. Biophys. Acta 894, 59–68.
18 Horton, P. and Lee, P. (1985) Planta 165, 37–42.
19 Siegenthaler, P.-A. and Déperty, F. (1976) Eur. J. Biochem. 61, 573–580.
20 Andersson, B., Åkerlund, H.-E. and Albertsson, P.-Å. (1976) Biochim. Biophys. Acta 423, 122–132.
21 Kyle, D.J., Kuang, T.-Y., Watson, J.L. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 89–96.
22 Laemmli, U.K. (1970) Nature 227, 680–685.
23 Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
24 Farchaus, J.W., Widger, W.R., Cramer, W.A. and Dilley, R.A. (1982) Arch. Biochem. Biophys. 217, 362–367.
25 Haworth, P. and Melis, A. (1983) FEBS Lett. 160, 277–280.
26 Larsson, U.K., Ogren, E., Oquist, G. and Andersson, B. (1986) Photobiochem. Photobiophys. 5, 255–262.
27 Haworth, P. (1983) Arch. Biochem. Biophys. 226, 145–154.
28 Aro, E.-M., Hundal, T., Carlberg, I. and Andersson, B. (1990) Biochim. Biophys. Acta 1019, 269–275.
29 Adir, N., Shochat, S. and Ohad, I. (1990) J. Biol. Chem. 265, 12563–12568.
30 Hundal, T., Virgin, I., Styring, S. and Andersson, B. (1990) Biochim. Biophys. Acta 1017, 235–241.
31 Mattoo, A.K., Marder, J.B. and Edelman, M. (1989) Cell 56, 241–246.
32 Somersalo, S. and Krause, G.H. (1989) Planta 177, 409–416.
33 Staehelin, L.A. (1976) J. Cell Biol. 71, 136–158.
34 Rubin, B.T., Barber, J., Paillotin, G., Chow, W.S. and Yamamoto, Y. (1981) Biochim. Biophys. Acta 683, 69–74.
35 Murphy, D.J. (1986) Biochim. Biophys. Acta 864, 33–94.
36 Barber, J., Ford, R.C., Mitchell, R.A.C. and Millner, P.A. (1984) Planta 161, 375–380.
37 Miller, M., Pedersen, J.Z. and Cox, R.P. (1988) Biochim. Biophys. Acta 943, 501–510.
38 Li, G., Knowles, P.F., Murphy, D.J., Nishida, I. and Marsh, D. (1989) Biochemistry 28, 7446–7452.
39 Cherry, R.J. (1979) Biochim. Biophys. Acta 559, 289–327.
40 Einstein, A. (1906) Ann. J. Physik. 19, 371–381.
41 Mitchell, R., Spillman, A. and Haehnel, W. (1990) Biophys. J. 58, 1011–1024.
42 Nicholson, D., Zara, S.J., Parsonage, N.G. and Barber, J. (1990) J. Theor. Biol. 145, 65–81.
43 Gal, A., Hauska, G., Herrmann, R.G. and Ohad, I. (1990) J. Biol. Chem. 265, 19742–19746.
44 Albertsson, P.A., Yu, S.-G. and Larsson, U.K. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.) Vol. II, pp. 835–838, Kluwer Academic Press, Dordrecht.