Biochimica et Biophysica Acta 851 (1986) 475–483
Elsevier

BBA 42140

Temperature-dependent changes in the antenna size of Photosystem II. Reversible conversion of Photosystem II_α to Photosystem II_β

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(Received 24 April 1986)

Key words: Thylakoid membrane organization; Photosystem II heterogeneity; Temperature effect; Photoinhibition; Electron transport; (Spinach chloroplast)

Changes in the organization of the chloroplast photosynthetic membrane at moderately elevated temperatures were detected by SDS-polyacrylamide gel electrophoresis, electron transport and fluorescence induction measurements and by subfractionation analyses of heat-treated thylakoids. The results revealed that above 30°C there is a dissociation of peripheral light-harvesting chlorophyll *a/b* complex (LHC II) from Photosystem II. This is followed by a migration of Photosystem II and a portion of its tightly bound LHC II to the Photosystem-I-rich non-appressed thylakoid regions. This lateral migration includes all identifiable Photosystem II polypeptides and not only the reaction centre or core polypeptides. Concomitantly, there is a conversion of Photosystem II_α to Photosystem II_β as judged from fluorescence induction measurements with the heated thylakoids. These results support the notion of Photosystem II_β being localized in the non-appressed thylakoids and possessing a smaller antenna size. All temperature-induced changes required a background level of monovalent cations, and were partially reversible upon lowering the temperature. In the 30–40°C temperature range, where most of the changes in the organization and location of Photosystem II occurred, there is very little inhibition of electron transport capacity. We postulate that the temperature-dependent separation of Photosystem II from LHC II is a physiological mechanism to prevent overexcitation and subsequent damage of Photosystem II at high-light intensities that are accompanied by elevated temperatures.

Introduction

The thylakoid membrane of higher plant chloroplasts is continuous but structurally differentiated into regions where the membrane is exposed to the stroma (non-appressed thylakoids) and grana stacks where the membrane regions are closely appressed [1]. Biochemical and ultrastructural data lent strong support to the idea that such structural differentiation is accompanied by a functional heterogeneity [2–5]. The ATP-synthase, the ferredoxin-$NADP^+$ oxido-reductase and Photosystem I (PS I) are located in the non-appressed regions, while most of Photosystem II (PS II)

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Abbreviations: Chl, chlorophyll; CPa, chlorophyll *a*-protein complex of Photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyluera; LHC II, light harvesting chlorophyll *a/b* complex of Photosystem II; Mes, 4-morpholinethanesulfonic acid; MGDG, monogalactosyldiacylglycerol; PS I, Photosystem I; PS II, Photosystem II; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Q_A and Q_B, primary and secondary quinone acceptors of Photosystem II.

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including its light-harvesting chlorophyll *a/b* antenna complex (LHC II) are located in the appressed thylakoid regions. A small fraction of PS II is found in non-appressed membrane fractions [2,3]. This type of PS II has been ascribed to PS II_β, while the major pool of PS II is localized in the appressed membranes of the grana and has been ascribed to PS II_α [3]. Apart from their lateral location the two types of PS II are thought to differ in terms of their kinetic properties [6,7], apparent mid-point potential of their primary electron acceptors [8,9], connectivity to the plastoquinone pool [10,11], as well as their relative DCMU sensitivity [12]. Moreover, the light-harvesting antenna size of $PSII_\beta$ is smaller than that of PS II_α [7,10].

More recent findings suggest that the model of lateral heterogeneity in the organization of the thylakoid membrane should not be regarded as static. Certain environmental stimuli can evoke controlled lateral migration of certain protein complexes between the non-appressed and appressed thylakoid regions. The most well-documented example is phosphorylation of the LHC II which results in its dissociation from the PS II core and its subsequent migration from the appressed to the non-appressed thylakoid regions [13–15]. This phosphorylation-induced migration of LHC II is thought to affect the energy distribution between the two photosystems under different light conditions [14,16,17]. Recently [18], we presented evidence, based on subfractionation of thylakoids at different temperatures, that reversible lateral rearrangements in the thylakoid membrane can also be induced by changes in temperature. Above 30°C the PS II and a portion of its tightly bound LHC II antenna migrate to the PS-I-rich nonappressed regions leaving free LHC II behind in the appressed thylakoid regions. A detachment of LHC II from PS II has also been suggested by fluorescence studies on intact leaves or isolated thylakoid membranes [19–22] and by freeze-fracture electron microscopy [23–25]. Of particular importance was the fact that such thermally induced changes were reversible after lowering the temperature [18]. It was therefore suggested that temperature-dependent changes in the organization of the light-harvesting apparatus may play a physiological role in photosynthesis by preventing overexcitation of Photosystem II under such high light intensities that are accompanied by elevated leaf temperatures [18,26].

In this study we have further analysed the organization of the thylakoid membrane at elevated temperatures by SDS-polyacrylamide gel electrophoresis, electron transport and fluorescence induction measurements. In particular we present biochemical and kinetic evidence demonstrating that thermally induced changes in the lateral organisation of the thylakoid membrane are accompanied by a reversible conversion of PS II_α to PS II_β.

Materials and Methods

Chloroplasts were prepared from spinach leaves grown at 20°C as previously described [27]. Thylakoid membranes were isolated after osmotic rupture, washing and resuspension in 10 mM sodium phosphate (pH 7.4)/5 mM NaCl/5 mM $MgCl_2$/100 mM sucrose. When thylakoids devoid of monovalent ions were desired the medium given above was replaced by 10 mM Tricine (pH 7.4)/5 mM $MgCl_2$/100 mM sucrose.

For the heat treatment thylakoids were suspended in glass tubes using one of the two suspension media to a chlorophyll concentration of 1000 μg Chl/ml and incubated in a water bath at the desired temperature (5–50°C) for 1–5 min. The heat treatment was terminated by transfer of the thylakoids to a reaction medium for immediate analysis of electron transport or fluorescence induction or by fragmentation in a Yeda-press, preheated to the desired temperature [18]. For reversibility studies, heated thylakoid samples were kept at 5°C for 45 min prior to analyses or fragmentation.

Fractionation of thylakoid membranes into stroma lamella vesicles representative of the non-appressed thylakoid regions and inside-out vesicles representative of the appressed thylakoid regions was performed by Yeda press treatment of the thylakoid suspensions and subsequent differential centrifugation and phase partition [27,28].

The chloroplast fluorescence induction kinetics were measured at 20°C with an Aminco spectrophotometer. The reaction mixture contained unfractionated thylakoid membranes (pretreated at

the indicated temperatures) at approx 20 μg Chl/ml, suspended in a medium containing 10 mM sodium phosphate (pH 7.4)/100 mM sucrose/5 mM NaCl/5 mM $MgCl_2$. The measurements were performed in the presence of 20 μM recrystallized DCMU. Actinic illumination of uniform field was provided in the green region of the spectrum [29]. Fluorescence emission at 690 nm was detected at a right angle with respect to the direction of the actinic beam. Signal recovery and storage of the kinetic traces was implemented with a Nicolet Signal Averager. Under these conditions, the area over the fluorescence induction curve is directly proportional to the amount of the primary quinone acceptor of PS II that becomes photoreduced [29,30]. The kinetic analysis of the area over the fluorescence induction curve was performed as previously described [6,7].

Rates of electron transport were measured with a Clark-type oxygen electrode using saturating red light. PS II activity was measured in the same medium as used for the fluorescence induction measurements. The Chl concentration was 10 μg Chl/ml and 0.2 mM phenyl-*p*-benzoquinone was used as electron acceptor. Whole-chain electron transport was measured in a medium of 40 μg Chl/ml in 40 mM sodium phosphate (pH 7.4)/0.6 mM NaN_3/1 mM NaCl and 0.12 mM methyl viologen. The electron-transport measurements were performed at 20° or 5°C.

Polypeptide analyses were performed by SDS-polyacrylamide gel electrophoresis using 12–22.5% gradient gels with the buffer system of Laemmli [31]. Chlorophyll concentration was determined according to Arnon [32].

Results

Temperature-dependent changes in the lateral location of PS II

The chlorophyll *a/b* ratio of inside-out vesicles isolated from thylakoids kept at 40°C was typically as low as 1.9 compared to 2.2 for vesicles from control thylakoids kept at 5°C (Table I). Concomitantly, the chlorophyll *a/b* ratio of the stroma lamellae vesicles from the heated thylakoids was lowered to 4.2 from the normally high ratio of 5.5 measured in control stroma lamellae vesicles. From analysis of chlorophyll-protein complexes in the various thylakoid subfractions [18], it was determined that such thermally induced changes are the result of a relative increase in the content of LHC II and of a decrease in the chlorophyll a protein complex of PS II (CPa) in inside-out vesicles, and a concomitant relative increase of both these complexes in the stroma lamellae vesicles. The reversible nature of these changes is shown in Table I, which gives the chlorophyll *a/b* ratios of the two thylakoid subfractions isolated from thylakoids heated to 40°C and subsequently incubated at 5°C for 45 min prior to fragmentation. The chlorophyll *a/b* ratios of these inside-out vesicles and stroma lamellae vesicles resemble the corresponding values for vesicles isolated from control thylakoids. Preliminary studies show that the reversibility is more complete if the thylakoids are kept at room temperature after the heating.

TABLE I

REVERSIBILITY OF TEMPERATURE-INDUCED LATERAL REARRANGEMENTS OF THE THYLAKOID MEMBRANE AS JUDGED BY CHLOROPHYLL *a/b* RATIOS IN THYLAKOID SUBFRACTIONS

Stroma lamellae vesicles and inside-out vesicles were isolated from thylakoids kept at 40°C for 1 min. Another sample was heated and then cooled and kept at 5°C for 90 min prior to fragmentation. Values given are Chl *a/b* ratios.

	Treatment of thylakoids prior to fragmentation		
	5°C	40°C	40°C/5°C
Inside-out vesicles	2.2	1.9	2.1
Stroma lamellae vesicles	5.5	4.2	5.2

Changes in the lateral distribution of the CPa complex as analysed by the mild SDS-polyacrylamide gel electrophoresis [18] do not reveal the nature of PS II polypeptides that are involved in the lateral rearrangements at elevated temperatures. To provide an answer to this question we performed denaturing SDS-polyacrylamide gel electrophoresis which resolved the individual polypeptides present in inside-out and stroma lamellae vesicles derived from normal and heated (40°C) thylakoids (Fig. 1). The polypeptide pattern of inside-out vesicles from the heated thylakoids are

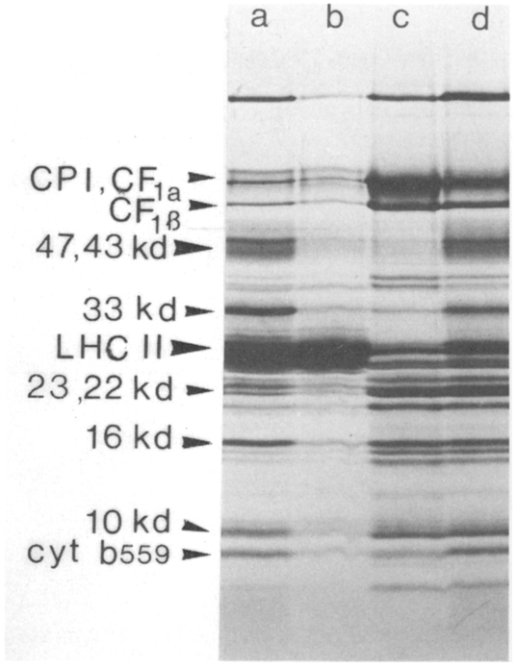

Fig. 1. SDS-polyacrylamide gelelectrophoresis of inside-out vesicles (a) and stroma lamellae vesicles (c) isolated from control (5°C) thylakoids, and inside-out vesicles (b) and stroma lamellae vesicles (d) isolated from thylakoids kept at 40°C prior to fragmentation. The samples were loaded on an equal chlorophyll basis. Note that inside-out vesicles derived from the heated thylakoids are dominated by the two apopolypeptides of LHC II (27 and 25 kDa). In this vesicle preparation there is a marked depletion of PS II polypeptides such as the 47 and 43 kDa apopolypeptides of the CPa complex, the 33, 23 and 16 kDa polypeptides of the oxygen evolving complex, the 9 kDa apopolypeptide of cytochrome *b*-559 and the newly identified 22 and 10 kDa polypeptides. For most of these PS II polypeptides as well as LHC II there is a concomitant increase in the stroma lamellae vesicles from the heated sample (d). For some of the PS II polypeptides, such as the 23 and 16 kDa polypeptides, this increase is hard to monitor due to comigrating PS I polypeptides (c, d). Note that the band at 110 kDa represents non-denatured CP I, which shows different stability to the SDS solubilization in the various subfractions, and can therefore not be used for quantification.

entirely dominated by the two major apopolypeptides of LHC-II, the 27 and 25 kDa polypeptides. Compared to the control inside-out vesicles, all PS II polypeptides, including the subunits of the PS II core complex [33], the extrinsic polypeptides of the water oxidating complex and other identifiable PS II polypeptides [34], were all substantially depleted. Concomitantly, the level of these PS II polypeptides were increased in the stroma lamellae vesicles from the heated thylakoids. It is important to note that the relative content of two LHC II apopolypeptides was also higher in the stroma lamellae preparation. Note that the residual amount of apopolypeptides of CF_1 and CP I in the inside-out vesicles does not change much upon heating. This is consistent with the interpretation that the presence of these polypeptides are due to contaminating right-side out vesicles and therefore not representative of the appressed thylakoid region [2].

From this polypeptide analysis it can be concluded that elevated temperatures cause a dissociation of LHC II from the entire PS II, which migrates to the non-appressed thylakoid regions together with a tightly bound complement of LHC II. The latter observation is consistent with our interpretation [18] that although the net amount of PS II is higher in stroma lamellae vesicles after heat treatment, the LHC II/CPa ratio remains unchanged. Thus, the migrating PS II units must have the same LHC II antenna size as the PS II_β units normally residing in the non-appressed thylakoid regions [3]. We therefore postulated that changes in the antennae organization following a heat treatment must involve the dissociation of PS II_α into free LHC II and PS II_β, and that the latter migrates from the grana to the non-appressed thylakoid regions [18].

Thermal interconversion between PS II_α and PS II_β

This postulation prompted a direct measurement of the relative amounts of PS II_α and PS II_β in thylakoids kept at different temperatures. This was implemented from the chlorophyll *a* fluorescence induction kinetics measured in the presence of DCMU with intact thylakoids that were incubated at different temperatures (Fig. 2a and b). In this approach, the area over the fluorescence induction curve (Fig. 2a) is directly proportional to the fraction of Q_A that becomes photoreduced [7,30]. In the control thylakoids the fluorescence induction shows that the slow β component accounts for only a small portion of the variable fluorescence. The proportion of PS II_β can be determined from the intercept of the linear phase with the ordinate in the semilogarithmic plot (Fig. 2b) of the area over the fluorescence curve (Fig.

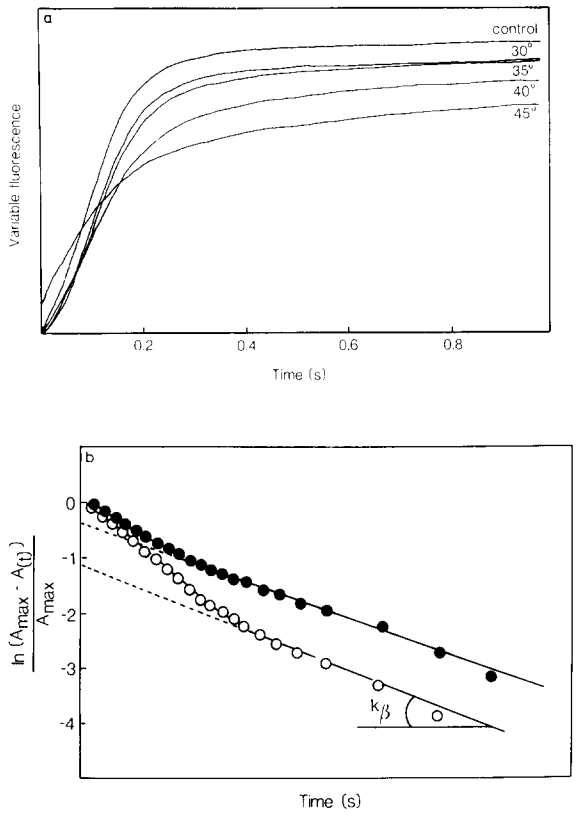

Fig. 2. (a) Kinetics of variable fluorescence of intact thylakoids heated for 1 min to various temperatures, and (b) first-order kinetic analysis of control (○) and the 42°C (●) heated thylakoids. The intercept of the linear phase with the ordinate gives the percentage of PS II_β for control and heated thylakoids.

2a). For control thylakoids kept at 5°C such an analysis revealed approx. 30% PS II_β centres and 70% PS II_α centres (Table II, Fig. 3). Thylakoids kept at 30°C show about the same ratio between the two forms of PS II. Above 30°C, however, the kinetics of the variable fluorescence were markedly altered so that the slow β component became dominant at the expense of the α component (Fig. 2a). Calculations from the semilogarithmic plots (Fig. 2b) revealed that after incubation at 40°C there is 67% PS II_β and only 33% PS II_α (Table II). At 45°C there is as much as 84% of PS II_β. It is important to note that although the relative proportion of PS II_α and PS II_β changed during sample incubation at temperatures greater than 30°C, the slope of the β component in the semi-

TABLE II

TEMPERATURE-DEPENDENT REVERSIBLE CONVERSION OF PS II_α TO PS II_β

Thylakoid were kept at the indicated temperature for 1 min and then transferred to a medium, 10 mM sodium phosphate (pH 7.4)/100 mM sucrose/5 mM $MgCl_2$, kept at 20°C and the fluorescence induction was immediately measured. For the reversibility experiments the heated samples were kept at 5°C for 45 min prior to the measurement. Kinetic analyses were made in order to determine the relative amount of PS II_α and PS II_β.

Temperature (°C)	PS II_α (%)	PS II_β (%)	PS II_α (converted %)	PS II_α (recovered %)
5	67	33		
35	42	58	37	
35/5	55	45		52
40	33	67	51	
40/5	49	51		47
45	16	84	76	
45/5	19	81		6

logarithmic plot (k_B) remained constant (Fig. 2b). This observation supports the notion that the antenna size of the newly formed PS II_β is the same as that of the original PS II_β. The conversion of PS II_α to PS II_β required as short as 1 min incubation of the thylakoids at the elevated temperatures. Longer periods of heating did not lead to much further increase in the concentration of PS II_β (not shown), but brought about a substan-

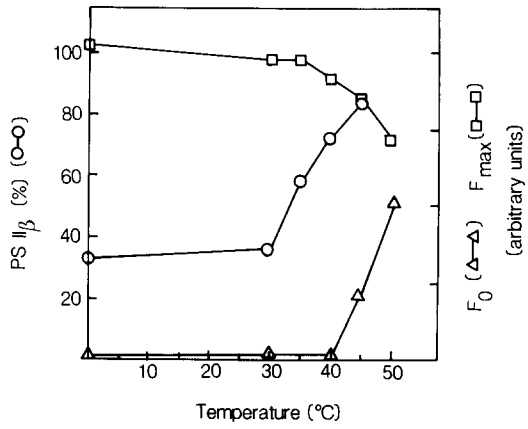

Fig. 3. Effect on PS II_β (○), F_0 (△) and F_{max} (□) of 1 min heating of intact thylakoids at various temperatures.

tial lowering of F_{max} and increase in F_0. A short period of heating was therefore chosen.

The fluorescence kinetic measurements lent strong support to the idea of a thermally induced conversion of PS II_α to PS II_β [18]. This interpretation is consistent with the observation that the fast α component of the fluorescence induction curve decreases upon heating leaves at 35°C [35]. Notably, the conversion of PS II_α to PS II_β is partially reversible upon lowering the temperature of the heated thylakoids (Table II). By placing the thylakoids heated to 35°C at 5°C for 45 min lowered the relative amount of PS II_β from 58% to 48%. The corresponding decrease for the 40°C sample was from 67% to 51%. Since the relative amount of PS II_β in the control thylakoid is 33%, there is an approx. 50% recovery of the lost PS II_α centres upon lowering the temperature of both the 35°C and the 40°C thylakoid samples. For incubation temperatures greater than 45°C the reversibility is almost entirely lost. Under our in vitro conditions, the heat treatment also induces secondary irreversible damage to the photosynthetic apparatus. This is reflected by an irreversible decrease in the F_{max} and increase of the F_0, particularly above 45°C (Fig. 3).

Effects of the increased temperatures on the electron transport

The partial reversibility of both the structural changes and the PS II_α/PS II_β interconversion are the main pieces of evidence in support of the hypothesis postulating that temperature induced changes may play a physiological role in photosynthesis [18,26]. Another prerequisite for such a postulation is that the temperature increase does not result in irreversible inhibition of the electron-transport capacity. We therefore analyzed both PS II and whole-chain electron transport after 1 min pre-heating of the thylakoids under exactly the same conditions as in the experiments described above. The activity measurements were performed at 20°C or 5°C. The latter temperature was included in order to minimize lateral diffusion, which might cause a reversion of the temperature induced structural changes during the activity measurements. However, both temperatures gave the same results.

Preheating up to 40°C does not cause much inhibition of the light-saturated PS II activity (Fig. 4a). This is in accordance with previous work [36], which shows that oxygen evolution is stable up to 40°C at neutral pH in the presence of chloride. Only at higher temperatures PS II activity is considerably decreased. This inhibition can be attributed to the release of manganese and of extrinsic polypeptides from the inner thylakoid surface [36]. Such inhibition at 45°C at the donor side of PS II probably explains the irreversible decrease in the F_{max} seen at the corresponding temperature (Fig. 3). Similarly, the whole electron-transport chain activity is only inhibited by pre-heating above 40°C (Fig. 4b), following the pattern of inactiva-

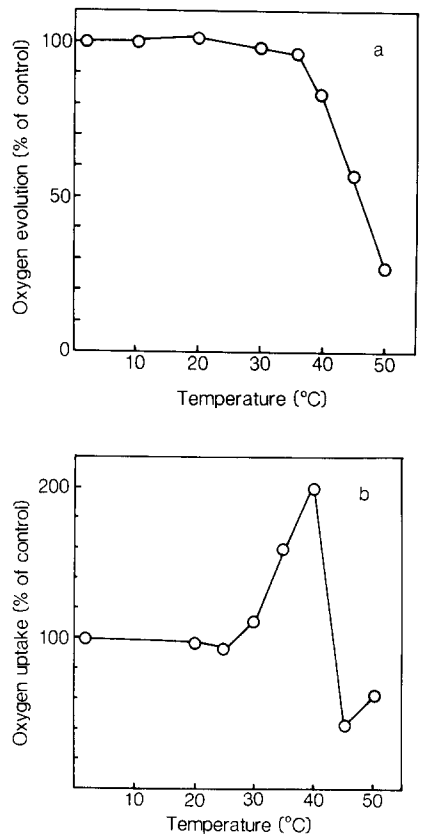

Fig. 4. Effects of heat treatment on (a) PS II mediated oxygen evolution in the presence of phenyl-*p*-benzoquinone (100% = 171 μmol O_2 per mg Chl per h) and (b) PS II plus PS I electron transport measured as methyl viologen catalyzed oxygen uptake (100% = 12.9 μmol O_2 per mg Chl perr h). Thylakoids were heated for 1 min at the indicated temperature and then transferred to the appropriate assay medium equilibrated to 20°C.

tion of the oxygen evolving complex. In the temperature range 30–40°C, however, there is a marked stimulation. This stimulation was seen also in the presence of the uncoupler CH_3NH_2, indicating that this was not an effect of a thermal uncoupling, but rather a result of changes in the rate-limiting step of PS I [37]. In the case of the PS II electron transport rates (Fig. 4a) these are independent of any uncoupling effects, since the measurements were performed in the presence of a class III electron acceptor [38,39].

In conclusion, the electron-transport activity measurements show that at 30–40°C where there is a pronounced conversion of PS II_α to PS II_β and the structural changes take place, there is only a minor inhibition of the PS II electron-transport capacity.

Cation requirement for temperature-induced changes in the PS II – LHC II association

During the course of our experiments we found that the thermally induced dissociation of LHC II from PS II required the presence of monovalent cations. In the experiments described above, the heat treatments were performed in a medium containing 20 mM Na^+ in addition to 5 mM Mg^{2+}. If the thylakoids were depleted of Na^+ by several washings and final suspension in 10 mM Tricine (pH 7.4)/5 mM $MgCl_2$ prior to the heat treatment, no dissociation of LHC II from PS II was detected (Table III). Instead, a destacking resembling that under low-ionic-strength conditions [40] took place. Thus, the inside-out vesicles isolated from the Na^+-depleted and heated thylakoids do not show any decrease in the chlorophyll *a/b* ratio. Moreover, the decrease in the yield of inside-out vesicles is less pronounced after heating and subfractionating in the absence than in the presence of the Na^+ (Table III) suggesting a more limited thermal destacking in the absence of Na^+. Heating the thylakoids in the absence of monovalent cations also drastically decreased the conversion of PS II_α to PS II_β as judged from the fluorescence induction measurements (not shown).

TABLE III

DEPENDENCE OF MONOVALENT IONS FOR THE INDUCTION OF LATERAL REARRANGEMENTS OF THE THYLAKOID MEMBRANE

Thylakoids were heated at 40°C in the presence or absence of 20 mM Na^+ prior to fragmentation. As control material, thylakoids kept at 5°C were used. In all samples, 5 mM $MgCl_2$ were present. Inside-out vesicles were isolated from the four samples and analyzed with respect to yield and chlorophyll *a/b* ratios.

Properties of inside-out vesicles	Conditions for thylakoid subfractionation			
	20 mM Na^+ + 5 mM Mg^{2+}		5 mM Mg^{2+}	
	5°C	40°C	5°C	40°C
Yield (%)	24	14	26	20
Chlorophyll *a/b*	2.3	1.9	2.4	2.3

Discussion

Our present results provide insight into the molecular events leading to the reorganization of the thylakoid membrane at moderately elevated temperatures. These include the dissociation of a pool of peripheral LHC II from PS II which retains a tightly bound pool of LHC II. PS II and the tightly bound LHC II migrate to the PS-I-rich non-appressed region leaving behind free LHC II in the appressed regions. These events which are partially reversible upon lowering of the temperature amount to a conversion of PS II_α to PS II_β. A structural rearrangement of the thylakoid membrane has previously been suggested from freeze-fracture studies [23–25]. In the study of Armond et al. [23], leaves were heated and thylakoids were isolated which showed changes in the exoplasmic fracture particles interpreted as a dissociation of LHC II from PS II. Their measurements provide a strong evidence that the thermally induced changes in the thylakoid membrane occur in intact leaves and not only with thylakoid membrane under in vitro conditions. In two other freeze-fracture studies Gounaris et al. [24,25] showed similar freeze-fracture particle changes. In addition, it was shown that there were thermally induced changes in the lateral distribution of freeze-fracture particles. These ultra-structural data was interpreted as PS II migrating to the non-appressed thylakoids, while free LHC II remained in the appressed regions [25] in accordance with our present biochemical study. Gounaris et al. [24,25] postulated that the changes reflected alterations in the interaction between the lipid MGDG and LHC II at elevated temperatures. A functional detachment of LHC II

from PS II at elevated temperatures and an increased PS I/PS II interaction have been indicated from several fluorescence studies [19–22] on heat-treated intact leaves or isolated thylakoid membranes. Interestingly, such temperature-induced fluorescence changes were reversible [21,22]. Our present study provides a direct molecular mechanism by which to explain these fluorescence changes.

Our data provide evidence for a temperature interconversion between PS II_α and PS II_β. The increase in PS II_β, as judged by the measurement of fluorescence kinetics, is parallelled by a dissociation of the LHC II from PS II and a concomitant increased fraction of PS II_β in the non-appressed regions. These results add support to the notion that PS II_β has a smaller antenna size and is located in the non-appressed thylakoid regions [3,7]. The conversion of PS II_α to PS II_β is not parallelled by a decrease in PS II activity. This would speak against PS II_β being inactive due to a disconnection between quinones at the PS II acceptor side [41]. However, we cannot exclude the possibility that the electron acceptor phenyl-*p*-benzoquinone used in the present study can reverse such a block. The reason for the ion dependence of the temperature induced PS II–LHC II dissociation is not obvious, but it is well established that protein–protein interactions are influenced by cations [16]. A similar requirement for cations is evident in the destacking of thylakoids, where a small concentration of monovalent ions is necessary to allow the appressed membrane surfaces to come apart [42]. This phenomenon has been discussed in terms of a competition between monovalent and divalent ions to screen the negative surface charges [16,42]. The stroma surrounding the thylakoid membrane contains both monovalent and divalent ions meaning that these temperature related changes can take place in vivo.

Another example of a controlled rearrangement in the lateral organization of the thylakoid membrane is phosphorylation of LHC II, which also results in a dissociation of peripheral LHC II from PS II [13–15]. However, the lateral rearrangements caused by elevated temperatures or phosphorylation differ (Fig. 5). In the case of phosphorylation LHC II moves to the non-appressed

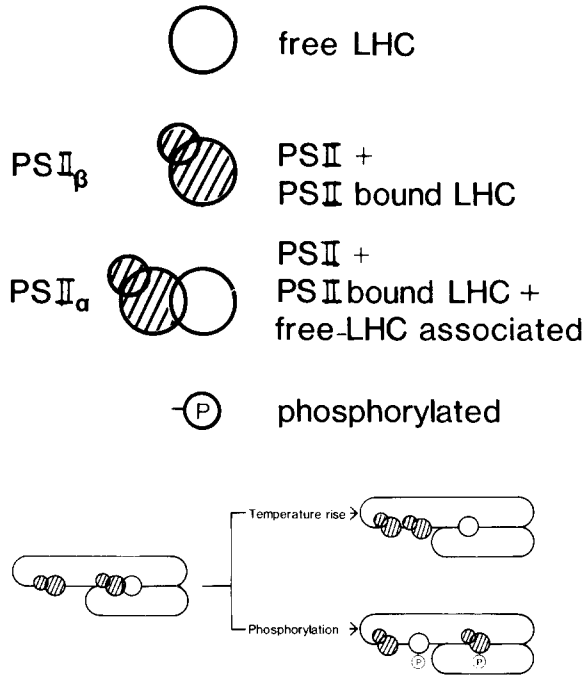

Fig. 5. Model comparing changes in the lateral distribution of PS II and LHC II between the appressed and non-appressed thylakoid regions evoked by elevated temperatures or phosphorylation.

membrane region, while the PS II and its tightly bound LHC II remain in the grana partition regions. At elevated temperatures, the situation is reversed, as free LHC II remains in the appressions and PS II and its tightly bound LHC II move to the non-appressed regions.

We postulate that the temperature-dependent membrane rearrangements may play a physiological role as is the case for the rearrangements following LHC II phosphorylation [14,16,17]. The reversibility of the temperature-induced changes and the fact that they appear to take place in the intact leaves all provide arguments for a physiological role. Moreover, at moderately elevated temperatures were the reorganisation is manifested inactivation of the electron-transport process is minimal or absent. The thermally induced separation of PS II away from LHC II may be a mechanism by which to prevent overexcitation and subsequent photodamage of the sensitive PS II reaction centre at high light intensities which

often is accompanied by high temperatures. Leaf temperatures in the 30–45°C range have been shown to occur under field conditions [43]. Photoinhibition of PS II is enhanced at low temperatures [44,45] at which the protective dissociation of PS II from LHC II would be obstructed due to decreased fluidity of the thylakoid bilayer. The thermally induced changes in the organization of the thylakoid membrane may therefore be a physiological mechanism to prevent photoinhibition and thereby help the plant cope with adverse light and temperature conditions in its environment.

Acknowledgements

We thank Ms. Sophie Bingsmark for skilfull technical assistance. P.M. acknowledges the receipt of a fellowship from the Nordic Council. A.M. was a Visiting Professor supported on a grant from the Swedish Natural Science Research Council. This work was supported by the Carl Trygger Foundation and the Swedish Natural Science Research Council.

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