Temperature-induced reversible migration along the thylakoid membrane of photosystem II regulates its association with LHC-II

Cecilia Sundby and Bertil Andersson

Department of Biochemistry, University of Lund, PO Box 124, S-221 00 Lund, Sweden

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Subfractionation of chloroplast thylakoids at elevated temperatures shows that above 35°C the photosystem II core and some of its light-harvesting complex (LHC-II) migrates laterally from the appressed thylakoid regions out to the non-appressed regions, leaving behind free LHC-II in the appressions. These structural rearrangements of the thylakoid membrane are largely reversible upon lowering the temperature and may play a physiological role in preventing overexcitation of photosystem II under high light intensities which normally induce elevated leaf temperatures.

Lateral migration *Light-harvesting complex (LHC-II)* *Photosystem II* *Photosynthesis regulation* *Thylakoid organization* *Thylakoid subfractionation*

1. INTRODUCTION

The photosynthetic apparatus is highly adaptive in order to maintain efficient energy conversion under a number of different environmental conditions. Many of the long-term adaptive responses due to variations in light intensity and quality involve changes in the composition and ultrastructure of the thylakoid membrane [1–4]. Typically, at high light intensities, there is an increased proportion of the PS I and CF_1-rich non-appressed thylakoid regions while at low light intensities there is an increased proportion of the PS II-rich appressed thylakoid regions [5]. Changes also occur in the lateral organization of the thylakoid membrane in response to short-term changes in the light regime. Several studies have shown that upon overexcitation of PS II, the LHC-II becomes phosphorylated and migrates away from the appressed thylakoid region into the PS I-rich non-appressed thylakoids [6–9].

Here, we present subfractionation evidence that changes in the distribution of chlorophyll-protein complexes along the thylakoid membrane occur not only in response to light but also to short-term changes in temperature.

Abbreviations: PS, photosystem; LHC-II, light-harvesting chlorophyll *a/b* complex of PS II; CF_1, coupling factor of thylakoids; chl, chlorophyll; SDS-PAGE, SDS-polyacrylamide electrophoresis

2. MATERIALS AND METHODS

Spinach thylakoids were prepared essentially as in [10] and 5-ml aliquots of the stacked thylakoid membranes suspended in 10 mM sodium phosphate, pH 7.4/5 mM NaCl/5 mM $MgCl_2$/100 mM sucrose at 1000 μg chl/ml were incubated for 5 min at the desired temperature (5–50°C) and thereafter subfractionated into stroma lamellae and inside-out vesicles representative of the non-appressed and the appressed thylakoids, respectively [11]. Thylakoid fractionation was performed according to [10] as modified in [12]. The thylakoids were passed only once through a Yeda press at 17.5 MPa at the desired temperature, rapidly cooled to 5°C, diluted 10-fold with 10 mM

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sodium phosphate, pH 7.4/5 mM NaCl/100 mM sucrose and centrifuged at 40000 × *g* for 30 min. The upper portion of the supernatants were collected and centrifuged at 100000 × *g* for 30 min to give stroma lamellae vesicles. The 40000 × *g* pellets were suspended in 10 mM sodium phosphate, pH 7.4/5 mM NaCl/100 mM sucrose and were subjected to phase partition following starch removal by centrifugation at 1000 × *g* for 10 min. The separation of inside-out vesicles from right-side-out vesicles was accomplished in a single partitioning step at 3.0°C (temperature critical), using a phase system composed of 5.6% (w/w) dextran T-500/5.6% (w/w) polyethylene glycol 3350/10 mM sodium phosphate, pH 7.4/5 mM NaCl/20 mM sucrose. By this simplified procedure a direct and rapid estimation of the relative amount of appressed and non-appressed thylakoids can be obtained [12].

Chl *a/b* determinations were made according to Arnon [13]. Analysis of the chlorophyll-protein composition was performed mainly according to [14,15] after mild SDS-PAGE at 0°C.

3. RESULTS

Fig.1 shows the changes in stacking behaviour of thylakoid membranes with increasing temperature. Yeda press treatment and phase partitioning of thylakoids at 5, 20 and 30°C give a constant yield of inside-out and stroma lamellae vesicles,

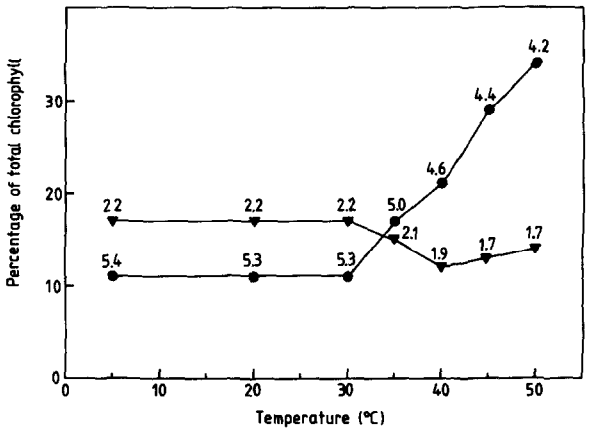

Fig.1. Chlorophyll yield and chl *a/b* ratios of stroma lamellae vesicles (●) and inside-out vesicles (▼) upon fragmentation of stacked thylakoids incubated for 5 min at various temperatures.

demonstrating that at these temperatures the proportion between appressed and non-appressed thylakoids is constant. Thylakoids at 35°C give a somewhat lower yield of inside-out vesicles and at 40–50°C this decrease is accentuated further. The decrease in yield of inside-out vesicles at elevated temperatures is accompanied by an increased proportion of non-appressed stroma lamellae vesicles. These observations provide evidence for a partial thermal destacking of thylakoids at temperatures above 35°C.

The chl *a/b* ratios of inside-out vesicles and stroma lamellae vesicles isolated from thylakoids at or below 30°C show constant and typical values of 2.3–2.4 and 5.3–5.4, respectively. Strikingly, thylakoids at 40°C yield inside-out vesicles with a chl *a/b* ratio as low as 1.9, a value that is decreased further to 1.7 at 45–50°C. At the same temperatures, the isolated stroma lamellae vesicles show chl *a/b* ratios well below 5. The relative composition of chlorophyll-protein complexes [14,15] was determined for the thylakoid subfractions isolated from thylakoids at different temperatures. Below 35°C, the relative composition of chlorophyll-proteins was similar to what is known for inside-out and stroma lamellae vesicles [15]. A distinct change occurred around 40°C, however. Fig.2 and table 1 both compare the relative composition of chlorophyll-proteins in inside-out and stroma lamellae vesicles isolated from thylakoids at 5 and 45°C. The most apparent change was that the percentage of PS II core complex ($CPa_1 + CPa_2$) in the inside-out vesicles was lowered from 14% in the 5°C sample to 5% in the 45°C sample. At the elevated temperature, the percentage of LHC-II ($LHCP^1 + LHCP^3$) increased from 60 to 66%. Thus, the LHC-II/PS II ratio in the appressed thylakoid regions at 45°C is 3-times as high as that at 5°C. The amount of free chlorophyll and PS I complexes ($CP1_a$ + CP1) was fairly constant at the two temperatures. The PS I content, which here is somewhat higher than in previous inside-out preparations [15] due to the simplified preparation procedure, is mainly a contamination of right-side-out vesicles [16]. Concomitant with the decrease in PS II content in the inside-out vesicles from the 45°C thylakoids the corresponding stroma lamellae vesicles showed an increased proportion of both PS II and LHC-II. Thus, the PS I/PS II ratio in the non-appressed

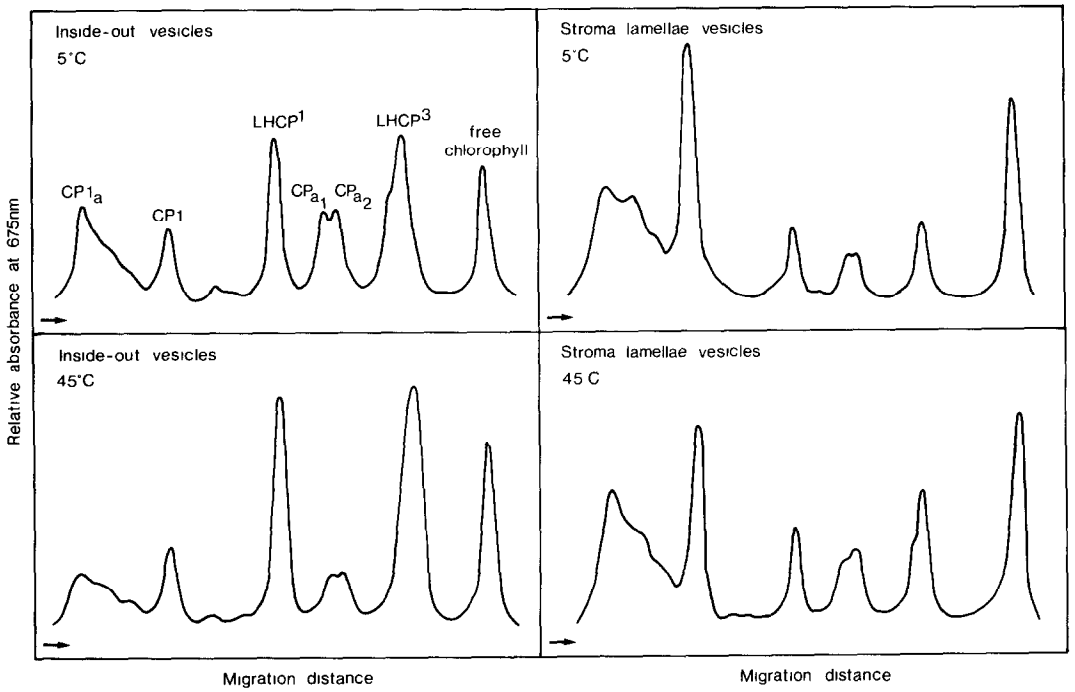

Fig.2. Gel scans at 675 nm comparing the relative proportion of chlorophyll-protein complexes in stroma lamellae and inside-out vesicles derived from thylakoids fragmented at 5 and 45°C.

Table 1

Chlorophyll-protein composition of inside-out and stroma lamellae vesicles isolated from thylakoids at 5 and 45°C

	Inside-out vesicles		Stroma lamellae vesicles	
	5°C	45°C	5°C	45°C
PS II	14	5	6	9
LHC-II	60	66	18	24
PS I	12	13	53	40
Free chl	14	16	23	27
$\frac{\text{LHC-II}}{\text{PS II}}$	4.3	13.2	3.0	2.7
$\frac{\text{PS I}}{\text{PS II}}$	0.9	2.5	8.9	4.4

The analysis of chlorophyll-protein content was performed by mild SDS-PAGE according to [14,15]. PS II = % ($CP_{a1} + CP_{a2}$), PS I = % ($CP1_a + CP1$), LHC-II = % $LHCP^1 + LHCP^3$) and free chl = % free chlorophyll. No $LHCP^2$ was resolved

thylakoid regions decreased from a normal value of 9 at 5°C to approx. 4 at 45°C. This indicates that an increased proportion of PS II has become intermixed with PS I in the non-appressed thylakoids. The LHC-II/PS II ratios in the stroma lamellae vesicles in the 5 and 45°C samples were fairly constant, indicating that the migrating PS II core is associated with a tightly bound pool of LHC-II.

Occasionally, a band containing mainly chl *a*, and with an apoprotein of 29 kDa, was resolved. This band, likely to be CP 29 [17], migrated together with PS II and its tightly bound LHC-II at the elevated temperatures. Moreover, in addition to the chlorophyll proteins of PS II the other subunits of the PS II core complex also participated in this lateral migration. This was particularly evident from a decreased content of apocytochrome *b*-559 (9 kDa) in the inside-out vesicles from the 40–50°C thylakoid samples (not shown). Similar qualitative changes to those shown for 45°C were observed for thylakoids incubated at all temperatures from 35 up to 55°C; quantitatively

Table 2

Reversibility of thermal-induced changes of the lateral distribution of PS II and LHC-II in the thylakoid membrane

Properties of inside-out vesicles	5°C	40°C, 5 min	40°C, 5 min + 5°C,90 min
Yield (%)	21	11	16
Chl *a/b*	2.2	1.9	2.1
PS II	11	8	10
$\frac{\text{LHC-II}}{\text{PS II}}$	4.7	7.0	5.7

Cooling of one of the 40°C thylakoid samples was done on ice immediately after 5 min incubation, and the sample was thereafter kept at 5°C for 90 min to allow lateral redistribution of chlorophyll-proteins prior to fragmentation. PS II = % ($CP_{a1} + CP_{a2}$), LHC-II = % ($LHCP^1 + LHCP^3$)

however, the changes increased with temperature within this interval.

As shown in table 2, the changes in lateral organization due to elevated temperatures were largely reversible upon lowering the temperature. Inside-out and stroma lamellae vesicles isolated from thylakoids incubated at 40°C for 5 min followed by incubation at 5°C for 1 h prior to fractionation showed about the same yield, chl *a/b* ratio and chlorophyll-protein composition as the corresponding fractions from the 5°C control thylakoids.

It should be noted that the electron transport capacity (measured from H_2O to methyl viologen at 20°C) of thylakoids incubated for 5 min at 40°C was not lower than that of control thylakoids.

4. DISCUSSION

Our results show that thylakoids subjected to elevated temperatures undergo a significant reorganization. There is a partial destacking where the reaction center of PS II and a tightly bound portion of LHC-II migrate from the grana appressions out into the PS I containing non-appressed regions. A part of LHC-II, which we suggest to be the peripheral pool of LHC-II, remains free in the appressions and thus becomes largely separated from both photosystems. The migrating PS II had the same LHC-II content as the PS II localized in the non-appressed regions of the control thylakoids (table 1), resembling PS II_β according to the definition of Anderson and Melis [18]. Therefore our results could be interpreted in terms of a thermal dissociation of PS II_α into free peripheral LHC-II and PS II_β. This lateral migration of PS II and tightly bound LHC-II contrasts with the situation following phosphorylation of thylakoid membranes where phosphorylated 'free' LHC-II, but not the PS II complex, migrates from the appressions into the non-appressed regions [7,9].

It should be mentioned that the thermal dissociation between LHC-II and the PS II core required approx. 15 mM monovalent cations in addition to 5 mM $MgCl_2$. If the monovalent ions were omitted and only 5 mM $MgCl_2$ was present at the increased temperatures, no inside-out vesicles with chl *a/b* ratios around 1.7 were formed. Instead, a destacking like that seen under low salt conditions was observed. This emphasizes that the lateral organization and assembly of the thylakoid complexes is regulated by an elaborate ionic control of surface charge repulsion [19].

A partial destacking of thylakoids and redistribution of freeze-fracture particles at elevated temperatures have been observed by electron microscopy [20,21] and it was suggested that the residual membrane adhesions contained LHC-II only. The biochemical data presented here strongly corroborate this suggestion. A reversible dissociation of LHC-II from the core of PS II is consistent with earlier fluorescence studies on whole leaves [22] and isolated thylakoids [23]. Moreover, a reversible development of state II at elevated temperatures has been described [24]. From very recent 77 K fluorescence measurements it was concluded that at temperatures above 35°C both the energy spillover between the photosystems and the absorption cross-section of PS I were increased [25].

Temperatures around 35–45°C are not unphysiological, since leaf temperatures up to 46°C have been recorded [26]. Such elevated temperatures are normally accompanied by high light intensities. The segregation of LHC-II from PS II could therefore be a short-term regulatory mechanism for avoiding overexcitation and

destruction of PS II at periods of very high light intensities during the course of a day, while still allowing an efficient electron transport to proceed at intermediate periods of weaker light intensities.

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